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Short communication

The immune-related fatty acids are responsive to CO_2 driven seawater acidification in a crustacean brine shrimp *Artemia sinica*

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ABSTRACT

The gradual increase of CO_2 concentration in the atmosphere, absorbed by the ocean surface water through air to sea equilibration termed ocean acidification (OA), leads to the decline of pH in seawater. It is not clear so far how the composition of fatty acids, particular the immune-related, in marine crustacean and the subsequent energy supply in marine ecosystem are affected by OA. The brine shrimp *Artemia sinica* is an open and common feed that provide essential fatty acids for mariculture. In this study, the fatty acids profiles of brine shrimp cultured under different lower pH levels of CO_2 driven seawater were investigated. The results showed a significant reduction of the proportion of total saturated fatty acids under the pH7.6 within one week. Meanwhile, the percentage of total monounsaturated fatty acids was significantly decreased at day 14 under pH7.8, and this percentage gave a significant increase of proportion within one week under pH7.6. Furthermore, the relative content of total polyunsaturated fatty acids (PUFAs) was found to be clearly increased with exposure to different seawater acidification at day 1, suggesting that the brine shrimp immune response was likely to be affected by acidified seawater as the PUFAs have been well known to be involved in immunomodulatory effects through alterations on cell membrane fluidity/lipid mediators and gene expression of cell signaling pathways. Notably, eicosapentaenoic acid and docosahexaenoic acid, which have essential effect on various physiological processes such as inflammatory cytokines production and cell structural stability, were strongly increased under two lower pH treatments within one week and with the significant increase at day 1 under pH7.6. These data clearly supported the hypothesis that OA might affect fatty acids composition, likely also the innate immunity, in crustacean and the subsequent energy transfer by food-chain system in the marine ecosystem.

1. Introduction

The increasing emission of carbon dioxide (CO_2) into the atmosphere by human activities leads to the change of dynamic balance of carbonate, in which nearly a third of atmospheric CO_2 is absorbed by the ocean surface water through air to sea equilibration. However, ocean CO_2 uptake further results in the reduction of pH level and carbonate chemistry alteration in seawater, which are commonly termed as ocean acidification (OA) (Orr et al., 2005). Seawater pH has already decreased by 0.1 units to pH 8.1 since preindustrial times. The Intergovernmental Panel on Climate Change (IPCC) predicted that the pH value of seawater will be dropped further by 0.3–0.4 units to pH 7.7 by the end of this century (Bopp et al., 2013) and OA has been presented as a serious challenge that faces marine organisms in the near future. A growing number of literature have shown that the direct or indirect impacts of predicted OA on marine organisms, including evident effects on development, reproduction and metabolism. For instance, the growth rate and calcification of many calcifying species were decreased under low seawater pH in the laboratory experiments

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(Beaufort et al., 2011; Doney et al., 2009; Kroeker et al., 2010). If the reducing trend of ocean pH continues, corals and some plankton will encounter with more difficulty in maintaining their external calcium carbonate skeleton (Orr et al., 2005). Meanwhile, OA can reduce fertilization rate of sea urchin *Heliocidaris erythrogramma* (Havenhand et al., 2008) and the cysts hatching rate of brine shrimp *Artemia sinica* (Zheng et al., 2015), and also break acid-base balance of the velvet swimming crab *Necora puber* (Spicer et al., 2007).

Fatty acids, the critical component of lipid, are the key energy sources transmitted in the marine food cycles, which are relatively conserved in the food chain transfer process and can be used as markers to trace the nutritional relationship between marine organisms (Parrish, 2009). Importantly, fatty acids are capable of modulating various cellular processes including the immune responses. Among diverse fatty acids composition, saturated fatty acids (SFAs) could stimulate the synthesis and release of inflammatory cytokines and trigger inflammatory response mediated by Toll-like receptors 4 (TLR4) (Rocha et al., 2016; Yang et al., 2015), while monounsaturated fatty acids (MUFAs) have suppressive or stimulative effects on the activation and differentiation of lymphocytes (Passos et al., 2016). In general, long chain PUFAs are known to modulate inflammatory responses by changing membrane fluidity, pattern of lipid mediators and inflammatory cytokines mediated by activation of key transcription factors associated with inflammatory gene expression (Calder, 2010). Brine shrimp is rich in protein, and contains various essential amino acids. Importantly, the crude fat content of brine shrimp is relatively high, of which unsaturated fatty acids are higher than saturated fatty acids. Brine shrimp is thus considered to be the most widely used live feeds in aquaculture on account of its special importance when rearing marine shrimp and larvae of some marine fish (Conceicao et al., 2010; Sorgeloos et al., 2001). A number of analysis on the nutritional components of brine shrimp has been carried out, but these studies are mainly related to variation in fatty acids profiles induced by various diary source and subsequent physiological changes in consideration to its key role as a good indicator of environmental stress (Chakraborty et al., 2007; Mana et al., 2014; Monroig et al., 2006). In order to better understand the physiological effects of OA on marine crustaceans, we conducted the qualitative and quantitative analysis on the content and changes of fatty acids by GC-MS technology in brine shrimp cultured under CO_2 driven acidified seawater, which could contribute to our understanding of OA on metabolism and immune functions by alteration of fatty acids in marine organisms, and further the impact of OA on the efficiency of energy transfer within marine food webs.

2. Materials and methods

2.1. Animal preparation

The *A. sinica* cysts (0.5 g) were hatched in 2 L tank (three tanks for each treatment) under different pCO_2 concentrations (780 ppm equivalent with pH7.8 and 1500 ppm equivalent with pH7.6 as the experimental group; 380 ppm equivalent with pH8.2 as the control group) at 26 °C in artificial sea water (28‰ salinity) with light intensity of 1000 lx. CO_2 was supplied by a central automatic CO_2-mixing-facility (CE100-3 model, Wuhan Ruihua Equipment Limited Company, China). Before incubation, the cysts were immersed in potassium permanganate solution (300 mg/L) for 5 min to be sterilized and then sufficiently washed using seawater. The spirulina powder was supplied twice a day for the larvae during the experimental period. The samples of *A. sinica* (approximately 20 mg) were collected at different developmental stages (post-hatch 1 day, 7 day and 14 day) with exposure to seawater acidification treatment for fatty acids extraction and GC-MS analysis (three independently experiments were performed).

2.2. Fatty acids extraction and derivatization

Extraction of crude fat from samples was performed according to the Floch extraction procedure (Folch et al., 1957). Briefly, the samples coupled with 50 μL of nonadecanoic acid were used as substitute. The standard working solution was added into the samples, and stored at 4 °C overnight. Next day, the samples were lapped by pellet pestle (Sigma-Aldrich, USA) and homogenized with 20 times volume of chloroform–methanol (chloroform: methanol = 2:1) mixture for lipid extraction. After centrifugation at 850 × g for 5 min, the supernatant was transferred to 50 mL storage bottles and homogenized with 3 mL of saturated normal saline using a pellet pestle on ice for at least 2 h. Then the upper liquid phase layer was removed and the lower layer organic phase layer was mixed with 1 mL of the chloroform: methanol: water (v: v: v = 3:48:47) solution. After discarding the upper layer, the extracts were then evaporated to dryness under a moderate nitrogen flow.

The residuum was added with 2 mL of boron trifluoride-methanol solution (14% of w/w) into each test tube followed by water bath at 60 °C for 5 min, then transferred into cold water immediately. After that, 4 mL of n-hexane and 3 mL of saturated normal saline were added into each tube with violent shake, and then the supernatant was transferred into 50 mL stripping tubes and eluted using 2 mL n-hexane followed by addition of 4 mL of n-hexane. The last step was repeated once. The extracts were evaporated to dryness under nitrogen, concentrated and transferred into 2 mL glass sample loops. The stripping tubes were washed with a small amount of n-hexane, and the washing liquid was transferred into glass sample loops. Washing was repeated three times. All the extracts were evaporated to dryness under nitrogen to 1 mL in water bath at 40 °C, transferred into sample vials, sealed with parafilm, and stored in darkness at −20 °C until the GC-MS analysis.

2.3. GC-MS analysis

GC/MS analysis was carried out using the gas chromatography coupled to the mass spectrometer (Agilent Technology, U.S.A). A constant (1.0 mL/min) flow of high purity helium was employed as the carrier gas. The temperature of the injection port was 260 °C with injection of 1 μL of sample. The whole heating process lasted for 33 min. The temperature of the column oven was: 1) 60 °C lasted for 2 min; 2) heated to 200 °C at 20 °C/min and lasted for 1 min; 3) heated to 210 °C at 10 °C/min and held for 2 min; 4) heated to 220 °C at 2 °C/min and held for 3 min; 5) heated to 240 °C at 5 °C/min and lasted for 2 min; 6) finally heated to 300 °C at 20 °C/min and lasted for 3 min. The transmission line was 300 °C. The mass spectrometer manipulated in electron impact (EI) mode, with the ion source at 230 °C, and the temperature of Quadrupole-MS was 150 °C. The samples were detected in SIM mode, and the detection voltage of the electron multiplier was 1600 V.

In this study, the content of fatty acids in *A. sinica* was calculated by external standard method (Folch et al., 1957). Fatty acids with the coefficient of determination of the standard curve $R^2 \geq 0.999$ were considered as reliable results, and the relative content of different fatty acids was calculated by the chromatographic peak area normalization method.

2.4. Statistical analysis

Results for all determinations were presented as means ± standard deviation (M ± SD). Statistical analysis was performed by

using two-way ANOVA with time and pH treatments as well as Fisher's least significant difference (Fisher's LSD) test.

3. Results and discussion

3.1. Composition of fatty acids in brine shrimp

To examine if there was any effect on the composition of fatty acids in brine shrimp cultured under CO_2 driven acidified seawater, most of the key fatty acids were primarily determined by GC-MS in control brine shrimp during their early and adult developmental stages under the normal pH8.2, which benefited further understanding on the variation of fatty acids profile in brine shrimp at different growth stages. In this study, 31 types of fatty acids, including SFAs, MUFAs and PUFAs, were identified, and 6 types of fatty acids were not detected with current detection method (S1 Table). From these identified fatty acids as shown in Table 1, the main fatty acids composition included 7 types of SFAs, 4 types of MUFAs and 5 types of PUFAs. SFAs mainly contained palmitic acid (8.54%–10.25%) and stearic acid (11.95%–19.3%); MUFAs were more with palmitoleic acid (6.22%–11.67%), oleic acid (12.58%–16.37%) and elaidic acid (12.79%–19.34%); while EPA (6.06%–9.78%) accounted for a relatively large proportion among PUFAs. Generally, the relative contents of unsaturated fatty acids were higher than those of SFAs. Meanwhile, the relative content of the determined fatty acids was altered during the development and growth stages of brine shrimp. The composition of total SFAs was significantly increased within one week followed by a marked decrease to initial level after 14 days under pH8.2 (Table 1). The observed increase of the total SFAs composition within the first week might reflect its importance during the early ontogenetic stages of brine shrimp. On the contrary, the relative percentage of total MUFAs was decreased substantially within one week and was increased slightly at day 14. Unlike the total SFAs and total MUFAs composition, the proportion of total PUFAs showed stable presence with almost no significant change over the entire experimental period (Table 1). It is generally believed that essential fatty acids such as EPA and DHA are the valuable essential fatty acids for growth, development and survival of juvenile larvae of crustacean and marine fish (Parrish, 2009). Actually, a number of studies have demonstrated that the fatty acids composition of brine shrimp strongly relied on the dietary sources such as microalgal and yeast due to their low capability of synthesizing ω-3 and ω-6 PUFA, particularly EPA and DHA (Vismara et al., 2003; Zhukova et al., 1998). Therefore, the fatty acids profiles of animals at different growth stages may vary with various diets. In addition, an earlier study has evaluated the difference of the fatty acids profiles of *Artemia nauplii* among the cysts, newly hatched nauplii and 24 h-old metanauplii, which indicated essentially unchanged composition percent of the fatty acids with an exception of the percentage of 16:0, 16:1 ω-7 and 20:5 ω-3 (Navarro et al., 1991); however, to our knowledge, no study has been reported about the variations of the fatty acids composition from brine shrimp at other developmental stages after 24 h-old metanauplii so far. Our study provides more data of the variations of the different fatty acids composition from brine shrimp at a later growth stage. Though some statistical difference of various fatty acids composition occurred in brine shrimp over the entire experimental period, the relative content of total SFAs, total MUFAs and total PUFAs, especially EPA and DHA, did not show clear difference until 14 day if compared to their initial levels, indicating no obvious difference of nutritional quality present in newly hatched nauplii and a later adult stage.

3.2. Effects on the fatty acids composition in brine shrimp under seawater acidification

To investigate the possible impacts of acidified seawater on the fatty acids composition of brine shrimp, the relative proportion of main fatty acids, including SFAs, MUFAs and PUFAs, to total fatty acids were further determined in brine shrimp exposed to lower pH levels (pH7.8 and pH7.6) at different sampling days as mentioned above. As shown in Table 1 and Fig. 1A, no significant difference occurred in the total SFAs composition of brine shrimp under the pH7.8 during the entire experimental period compared with control treatment. However, the ratio of the total SFAs composition was significantly decreased within one week under pH 7.6. Notably, the relative content of stearic acid was significantly reduced in brine shrimp when exposed to the pH7.6 seawater at both the day 1 and day 7. Stearic acid has been proved to stimulate the synthesis and release of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α) in human trophoblast cells (Yang et al., 2015). Importantly, the

Table 1
The proportion of main fatty acids composition of *A. sinica* in control group and seawater acidification groups at different developmental stages. The % of total fatty acids did not include some minor components due to the limitation of detection method. The asterisk indicates significant difference compared with those of controls ($^{*}p < 0.05$, $^{**}p < 0.01$).

Fatty acids	1st day (%)			7th day (%)			14th day (%)		
	pH 8.2	pH7.8	pH7.6	pH 8.2	pH7.8	pH7.6	pH 8.2	pH7.8	pH7.6
Myristic acid (C14:0)	1.25 ± 0.04	1.30 ± 0.06	2.27 ± 0.15**	2.06 ± 0.20	2.06 ± 0.15	1.99 ± 0.23	1.85 ± 0.07	1.80 ± 0.04	1.36 ± 0.05*
Pentadecanoic acid (C15:0)	0.59 ± 0.01	0.56 ± 0.01	0.51 ± 0.01	0.66 ± 0.09	0.73 ± 0.06	0.76 ± 0.02	0.79 ± 0.28	0.98 ± 0.21	0.56 ± 0.01
Palmitic acid (C16:0)	8.58 ± 0.06	11.14 ± 0.15*	7.05 ± 0.20	10.25 ± 1.08	9.29 ± 0.80	8.60 ± 0.28	8.54 ± 0.22	7.15 ± 1.68	11.51 ± 0.11**
Heptadecanoic acid (C17:0)	1.86 ± 0.03	2.92 ± 0.05**	1.37 ± 0.07	2.38 ± 0.32	2.39 ± 0.13	2.26 ± 0.50	2.30 ± 0.07	3.38 ± 0.14**	2.97 ± 0.03*
Stearic acid (C18:0)	15.28 ± 0.09	12.90 ± 0.16	6.87 ± 0.43**	19.30 ± 0.44	17.37 ± 0.85	14.96 ± 1.50**	11.95 ± 2.05	12.83 ± 0.24	13.32 ± 0.15
Arachidic acid (C20:0)	1.59 ± 0.33	1.08 ± 0.42	0.87 ± 0.41	3.09 ± 0.63	2.44 ± 0.70	1.67 ± 0.74	2.72 ± 0.32	5.02 ± 0.35**	0.87 ± 0.08*
Benhenic acid (C22:0)	2.43 ± 0.34	1.33 ± 0.53	1.05 ± 0.52	3.82 ± 0.79	3.00 ± 0.85	2.02 ± 0.67**	3.42 ± 0.24	6.22 ± 0.95**	1.06 ± 0.08*
Total SFA	**31.58 ± 0.76**	**31.30 ± 0.96**	**19.93 ± 0.36****	**41.55 ± 0.51**	**37.29 ± 3.54**	**32.26 ± 3.35****	**31.57 ± 2.62**	**37.38 ± 2.21**	**31.50 ± 0.38**
Palmitoleic acid (C16:1)	11.10 ± 0.62	10.25 ± 0.13	14.67 ± 0.60**	6.22 ± 0.09	6.78 ± 0.01	10.52 ± 0.65**	11.67 ± 2.19	7.68 ± 0.55**	10.08 ± 0.32
Oleic acid (C18:1)	16.15 ± 0.40	14.78 ± 0.33	23.40 ± 0.14**	12.58 ± 1.55	14.42 ± 2.39	15.46 ± 0.51	16.37 ± 1.96	10.20 ± 0.84**	14.72 ± 0.18
Elaidic acid (C18:1T)	19.34 ± 0.16	16.50 ± 0.76	13.16 ± 0.38**	13.99 ± 1.13	15.58 ± 2.64	18.88 ± 0.53**	12.79 ± 0.30	9.06 ± 0.98*	16.80 ± 0.15*
Eicosenoic acid (C20:1)	1.74 ± 0..30	1.16 ± 0.41	1.14 ± 0.38	3.24 ± 0.63	2.61 ± 0.68	1.90 ± 0.30*	2.91 ± 0.43	5.16 ± 0.20**	0.96 ± 0.07**
Total MUFA	**48.32 ± 0.88**	**42.67 ± 0.91***	**52.21 ± 0.69**	**36.03 ± 2.14**	**39.38 ± 4.35**	**46.75 ± 0.32****	**43.74 ± 0.96**	**32.10 ± 1.47****	**42.72 ± 0.69**
Linoleic acid (C18:2)	2.64 ± 0.09	10.09 ± 0.32**	5.86 ± 0.18**	3.69 ± 0.54	3.68 ± 0.51	3.67 ± 0.04	4.25 ± 0.82	5.85 ± 0.66*	10.29 ± 0.21**
α-Linolenic acid (C18:3)	2.23 ± 0.52	3.46 ± 0.24	4.31 ± 0.16	4.26 ± 0.81	3.63 ± 0.91	0.00 ± 0*	4.59 ± 0.53	7.19 ± 3.04	3.30 ± 0.10
Eicosatrienoic acid (C20:3)	3.34 ± 0.29	2.62 ± 0.45	2.56 ± 0.30	5.32 ± 0.88	4.95 ± 0.74	4.64 ± 0.06	4.99 ± 0.17	8.27 ± 1.08**	2.38 ± 0.05**
EPA (C20:5)	9.78 ± 0.59	6.76 ± 0.67**	10.21 ± 0.36	6.06 ± 0.21	9.93 ± 0.80**	10.42 ± 0.33**	9.15 ± 0.67	6.63 ± 0.33**	7.64 ± 0.42
DHA (C22:6)	2.10 ± 0.31	3.16 ± 0.43	4.7 ± 0.62**	3.09 ± 0.28	1.15 ± 0.29**	2.26 ± 0.60	1.71 ± 0.36	2.58 ± 0.43	2.20 ± 0.34
Total PUFA	**20.10 ± 0.01**	**26.11 ± 1.67****	**27.74 ± 0.99****	**22.42 ± 2.30**	**23.34 ± 1.07**	**20.99 ± 0.25**	**24.69 ± 0.43**	**30.52 ± 3.36****	**25.68 ± 0.45**

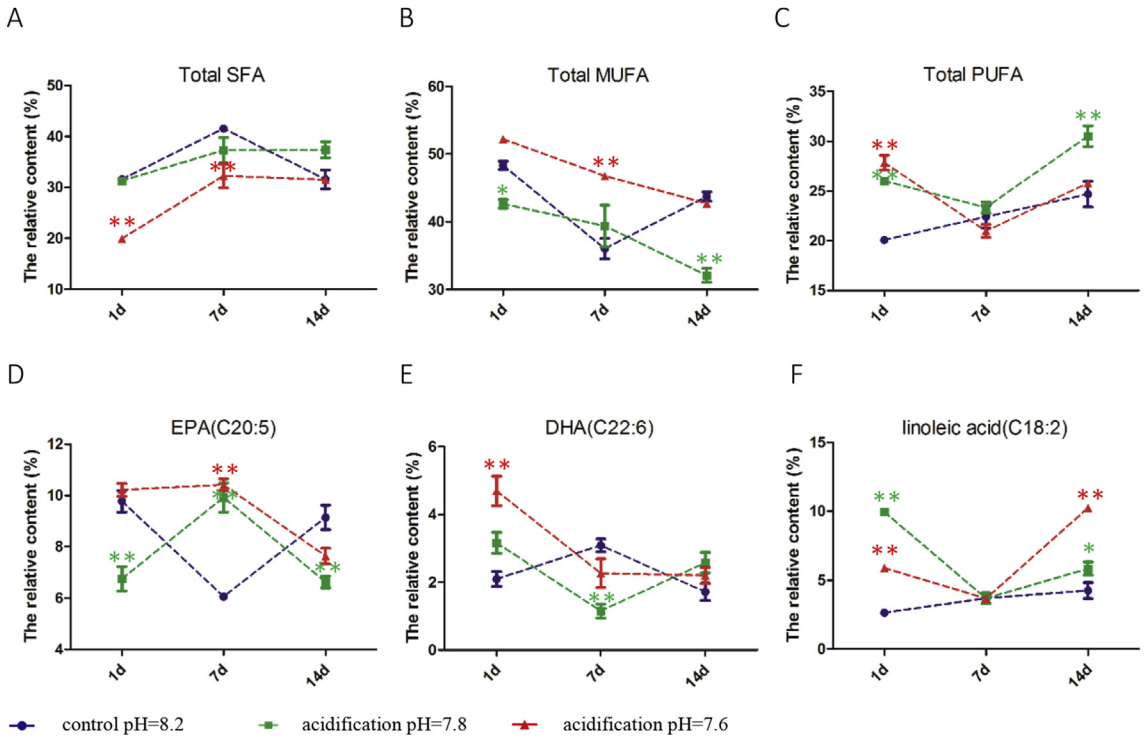

Fig. 1. The alteration of total SFAs, MUFAs and PUFAs (including EPA, DHA and linoleic acid) in brine shrimp *A. sinica* cultured under different pH levels of CO_2 driven seawater acidification. This experiment was repeated for three times. The asterisk indicates significant difference compared with those of controls (*$p < 0.05$, **$p < 0.01$).

stimulatory effects of stearic acid on the secretion of monocyte chemo-attractant protein-1 (MCP-1) from adipose tissue macrophages are mediated via Toll-like receptor-4 and NF-κB pathway (Schaeffler et al., 2009). These data highlight an essential molecular mechanism by which stearic acid can link metabolism with innate immunity. In consideration to that crustacean solely has innate immunity, further study will be necessary to determine if the reduction of stearic acid was associated with immune protection against pathogen infection during development and growth in the brine shrimp under stress of seawater acidification.

The relative percentage of total MUFAs was significantly reduced at day 14 in the pH7.8 seawater group but without clear change within one week if compared to the control group. After treatment with pH7.6 of seawater, the proportion of total MUFAs exhibited a significant increase at day 7 (Fig. 1B). Furthermore, the relative content of oleic acid was substantially increased under the lower pH7.6 at day 1, meanwhile palmitoleic acid was found to be markedly increased within one week (Table 1). A study in human has shown that palmitoleic acid could promote a suppressive effect on lymphocyte proliferation by reducing the secretion of Th1 and Th17 cytokine as well as the expression of co-stimulatory molecule CD28 (Passos et al., 2016). Conversely, oleic acid was shown to stimulate lymphocyte proliferation associated with interleukin-2 production and Th2 cells response in the same study (Passos et al., 2016). Since lack of acquired immune system and few study on this topic in crustacean, whether the alteration of palmitoleic acid and oleic acid are associated with immunity in brine shrimp are poorly understood.

The relative percentage of total PUFAs was clearly increased after one day post acidification treatment with pH7.8 and pH7.6 (Fig. 1C). Omega-3 PUFAs have been shown to modulate anti-inflammatory and immunomodulatory effects via a variety of ways, including decreasing generation of pro-inflammatory cytokines and arachidonic acid-derived eicosanoids, increasing generation of EPA-derived eicosanoids and EPA- and DHA-derived resolvins (Calder, 2009). On the other hand, change of PUFAs contents in membrane phospholipid would influence immune cell function through alterations in membrane fluidity and the pattern of lipid mediators as well as effects on cell signaling pathways (Calder, 2009). It was notable that the increase of relative content of EPA was occurred unexpectedly among different seawater acidification treatments within one week (Fig. 1D), and the relative content of DHA was also significantly increased under pH7.6 at the first day but without clear change afterward (Fig. 1E). A balanced ratio between EPA and DHA in phospholipids of biomembrances is assumed to be essential to maintain fluidity, and exceeding particular ranges particularly high EPA and low DHA levels may lead to lacking vitality and high mortality in larval fish (Rainuzzo et al., 1995; Watanabe and Kiron, 1994). The relative high ratio between EPA and DHA was observed under both pH7.8 and pH7.6 within one week in this study. Combined with our previous study, the survival rate of brine shrimp was declined when exposed to lower pH7.8 and pH7.6 (Zheng et al., 2015), which may suggest a potential correlation between animal survival rate and unbalanced ratio of EPA/DHA that needs further more investigation. Apart from the role of EPA and DHA as principle energy substrate and structural constituents of phospholipids in cell membranes, EPA and DHA have been demonstrated to affect immune function of a wide range of cell types such as endothelial cell, monocytes and macrophages in human through a variety of mechanism, including alteration of adhesion molecular expression and production of inflammatory cytokines (Calder, 2013). Furthermore, EPA and DHA are also considered as inflammation-resolving by producing lipid mediators, including eicosanoids, resolvins and neuroprotectins (Kremmyda et al., 2011; Serhan et al., 2008). Therefore, the mechanism of EPA and DHA on various physiological processes in brine shrimp *A. sinica*, particularly on immune response, should be further focused on. Additionally, linoleic acid (LNA) was markedly

increased with the same alteration tendency of PUFAs (Fig. 1F), although the relative content of α-linolenic acid (ALA) was not significantly changed during the entire experimental period. In juvenile grass carp *Ctenopharyngodon idella*, optimal dietary ALA/LNA ratio could enhance lysozyme and acid phosphatase activities, and up-regulate the transcripts of antimicrobial peptides and anti-inflammatory cytokines, yet suppress the transcripts of signal molecular such as IκB kinase β and NF-κB p65, and pro-inflammatory cytokines in gill (Zeng et al., 2017). Additionally, another study has also been shown optimal dietary ALA/LNA could improve mucosa immunity in the intestine of juvenile grass carp *Ctenopharyngodon idella* via significantly increased antibacterial compounds (such as acid phosphatase, lysozyme, and complement) and anti-inflammatory cytokines mRNA abundance as well as suppressed the mRNA levels of IL-1β, IL-8, TNF-α and IFN-γ2 (Zeng et al., 2016). These data suggested that optimal dietary ALA/LNA ratio is likely to be involved in innate immunity. Interestingly, the relative content of LNA in brine shrimp was markedly increased at day 1 and 14 post acidification treatment with both pH7.8 and pH7.6 in our present study. Therefore, we speculate that the variation of LNA might also affect immunity in brine shrimp when exposed to ocean acidification, which will be further investigated near future.

It should be noted that fatty acids, as a kind of important nutrition indicators, plays a very important role in the process of nutrition transferring dynamics of the ecosystem. Current understanding of OA effect on marine organism is mainly limited to the response of a single species, while the aftermath of OA effect on marine food web is still lacking. OA may indirectly affect consumers by altering the nutritional quality of primary producers or lower trophic level, further causing far-reaching consequences for marine food webs. For example, the fatty acids concentration and composition of the diatom *Thalassiosira pseudonana* has been significantly changed by OA, which led to the restriction of growth and reproduction of the copepod *Acartia tonsa* feeding on these diatoms as well as the energy supply for higher trophic level (Rossoll et al., 2012). In addition, a study by Gemma Cripps et al. has shown that the carbon trophic transfer efficiency was declined in populations from phytoplankton to zooplankton when exposed to the combined treatment between elevated *p*CO2 and feeding on the mixed prey reared under increased *p*CO2 (Cripps et al., 2016). In our present study, the brine shrimp was fed with spirulina powder, instead of the common microalgae such as *Chlorella regularis*, followed by the determination of its fatty acids composition. Hence, the effects of OA on the nutritional quality of brine shrimp feeding on phytoplankton cultured under seawater acidification as well as the transferring of energies are necessary to be further investigated for the elucidation of the OA effect on the nutrition or energy transfer in marine ecosystem. Besides, no difference was found in the fatty acids composition of the calanoid copepod *Eurytemora affinis* cultured under lower pH of CO_2 driven seawater (Almén et al., 2016). Nevertheless, the proportion of total PUFA in the copepod species, *Acartia bifilosa* and *Eurytemora affinis* was significantly decreased but without change of specific PUFA in *A. bifilosa* or *E. affinis* over the entire experimental period under high and low CO_2 treatments (Bermúdez et al., 2016). In addition, a study have shown that the larval stages of *Paracentrutos lividus* and *Lytechinus variegatus* are very sensitive to small changes of pH in seawater, in which the negative effects such as smaller or abnormal larvae and lighter-weight juveniles were evident at pH7.8 (Passarelli et al., 2017). Meanwhile, the sublethal effects of OA on metabolism are also evident for coral larvae (Munday et al., 2009). The fatty acids composition of different marine organisms to OA was varied due to their capabilities to compensate for the increase of CO_2 in seawater (Wittmann and Pörtner, 2013). However, the mechanism to this acidification stress is unknown which are still worthy of furthermore investigations.

4. Conclusion

All of that significant difference indicates that seawater acidification does have impact on the content of fatty acids in a crustacean *A. sinica* as a key trophic level in marine food webs, further effect may occur on the energies transmitted in the marine ecosystem. Simultaneously, change in fatty acids composition may also influence immunity in brine shrimp. Anyway, the effect of environmental variation on metabolism is an extremely complex process in marine organisms affected by multiple environmental stressors not only by CO_2 emissions but also with temperature and other factors. Therefore, more field research should be carried out to evaluate the potential ecological interactions between different environmental stressors and marine organisms' physiology, which may further benefit the understanding of effect on marine organism by climate change.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dci.2017.12.022.

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