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A marine secondary producer respires and feeds more in a high CO_2 ocean

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ABSTRACT

Climate change mediates marine chemical and physical environments and therefore influences marine organisms. While increasing atmospheric CO_2 level and associated ocean acidification has been predicted to stimulate marine primary productivity and may affect community structure, the processes that impact food chain and biological CO_2 pump are less documented. We hypothesized that copepods, as the secondary marine producer, may respond to future changes in seawater carbonate chemistry associated with ocean acidification due to increasing atmospheric CO_2 concentration. Here, we show that the copepod, *Centropages tenuiremis*, was able to perceive the chemical changes in seawater induced under elevated CO_2 concentration (>1700 μatm, pH < 7.60) with avoidance strategy. The copepod's respiration increased at the elevated CO_2 (1000 μatm), associated acidity (pH 7.83) and its feeding rates also increased correspondingly, except for the initial acclimating period, when it fed less. Our results imply that marine secondary producers increase their respiration and feeding rate in response to ocean acidification to balance the energy cost against increased acidity and CO_2 concentration.

1. Introduction

The ocean is known to absorb about one million tons of CO_2 per hour and have been acidified by 30% (increase in H^+ concentration) since the industrial revolution and will be further acidified by 150% by the end of 2100 (Caldeira and Wickett, 2003) under the "business as usual" CO_2 emission. Chemical changes associated with the ocean acidification include increased concentrations of H^+ and HCO_3^- and decreased levels of CO_3^{2-} and $CaCO_3$ saturation state, which negatively influence calcification of most marine calcifiers (Riebesell et al., 2000; Gao and Zheng, 2010; Beaufort et al., 2011). In addition, ocean acidification is known to influence olfactory functions of fish (Munday et al., 2009; Dixson et al., 2010) and can alter thermal windows of marine animals (Pörtner and Farrell, 2008).

Elevated seawater pCO_2 level is known to affect marine benthic and pelagic animal's development, reproduction and metabolisms (Kurihara and Ishimatsu, 2008; Widdicombe and Spicer, 2008). Decreased pH was shown to reduce the sperm flagella motility of reef invertebrates (Morita et al., 2010) and echinoderm larval feeding efficiency (Dupont and Thorndyke, 2008). Additionally, low pH may affect respiratory carbon loss in plankton (Wu et al., 2010). The effects of low pH on marine animals are often due to acidosis and hypercapnia generated in the intracellular space (Widdicombe and Spicer, 2008), which will disturb the balance of acid–base (Pörtner et al., 2004) and result in influences on behavioral performance (Thistle et al., 2007) and physiological processes (Roos and Boron, 1981), such as neural signals (Waggett and Buskey, 2008), development, reproduction (Kikkawa et al., 2004), metabolism and even gene expressions (Pörtner et al., 2010).

Regulation of body acid–base is somewhat energy dependent and low pH induced acidosis or hypercapnia can be expected to affect the energy acquisition and allocation (Whiteley, 2011). Here we hypothesize that the increased partial pressure of CO_2 and acidity of seawater associated with ocean acidification may affect copepods' respiration to cope with the chemical changes, and hence it would mediate its feeding rate to meet the energy demand. We chose a costal water calanoid copepod, *Centropages tenuiremis*, to test this hypothesis. This species is a major dominant copepod in the coastal South China Sea and plays an important role in the coastal ecosystem (Wang et al., 2005). In coastal waters where pH usually fluctuates during a daily cycle, the planktons usually experience wider ranges of pH compared to offshore species (Yamada and Ikeda, 1999). Therefore, we judge that response of a coastal copepod to changed seawater chemistry can be considered representative for offshore species that experience fewer changes in pH and should be more sensitive to changes in seawater carbonate system.

2. Materials and methods

2.1. Zooplankton sampling and pre-culture

C. tenuiremis individuals were obtained at night through horizontal hauling with a medium plankton net (mesh diameter

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0.112 mm) from surface water at the center of Xiamen Bay (24° 26.778′N, 118° 02.36′E) during the period of November 10, 2009 to 28 January, 2010, when the SST ranged 18–24 °C (measured with mercurial thermometer) and salinity averaged 26 (measured with a hand-hold refractometer, ATAGO, S/Mill-E, Japan). Samples were quickly transported to the laboratory within 60 min. Actively moving *C. tenuiremis* individuals were selected, nursed in 5 L beaker (about 300–500 individuals) for 12 h and fed with the diatom *Phaeodactylum tricornutum* at a concentration of 5×10^4 cells ml^{-1} under 20 °C and 20 μmol m^{-2} s^{-1} of cool-white light (LD cycle 12:12). *P. tricornutum* (CCMA 106) were obtained from Center for Collections of Marine Bacteria and Phytoplankton (CCMBP) of the State Key Laboratory of Marine Environmental Science (Xiamen Univ.) and was semi-continuously (partial renewal every 24 h) cultured at its exponential phase under 70 μmol m^{-2} s^{-1} (LD cycle 12:12) at 20 °C.

2.2. Behavioral response test

To test if the copepod is able to sense the chemical changes associated with ocean acidification, a flume made of transparent polymethyl methacrylate with two inlets respectively connected to ambient or high CO_2-equilibriated seawater were used (Fig. 1). Seawater was collected from the sampling site, filtered (0.22 μm) and sterilized by autoclaving. The seawater equilibrated with pure CO_2 (99.95%) was mixed with the sterilized seawater to obtain desired pH levels (7.00, 7.60, 7.80 and 8.15) (Gattuso et al., 2010). The pH was measured with a pH meter (Mettler Toledo DL15 Titrator, Sweden) that was calibrated with standard NBS (National Bureau of Standards) buffer solution (Hanna). The two kinds of seawater were sealed in two transfusion bags, and their flow rates (ca. 5 ml min^{-1}) were adjusted with a manual controller integrated with the bags. The bags with the seawater hung on the two sides of choice channel were switched frequently during the experiment in order to eliminate side-preference if there would be any. The CO_2-acidified and ambient seawaters can be clearly distinguished in the mixed area based on the test using dyed water ($KMnO_4$ solution as color indicator). Two meshes (mesh size 0.15 mm) were inserted between the in and out flow to form a choice area (length 3.5 cm, width 2 cm) and to eliminate the eddy. Pre-cultured copepods (as described above) were directly used in behavioral sensitivity test. For each test, 8–20 individuals were released to the mixed water area, and the individuals in acidified or ambient seawater areas were counted after copepods were released into the choice area for 6 min (the time is enough for the copepod to distinguish to the choice areas based on preliminary tests). Then, the number of copepods in the two pH areas was counted immediately. The sensitivity to changes in acidity was determined as the proportions of the individuals at the areas of differed acidity. Each test used freshly collected copepod individuals that were threw away after each use. The test was carried out in darkness to eliminate the influence of light and at 20 °C, which was similar to the ambient temperature at sampling site.

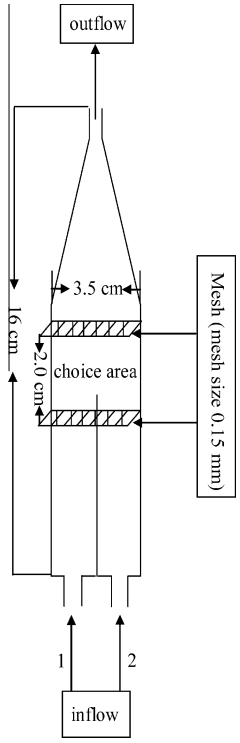

Fig. 1. The draft of two channel flume (length 16 cm, width 3.5 cm, height 3 cm) used in pH sensitivity test. The numbers "1" and "2" are two inlets that were separately connected to the transfusion bags which store the seawater. Two meshes were put in the channel to form a choice area (3.5 cm × 2 cm) in which the high- and low-CO_2 water can be distinguished based on the dye test. Not drawn to scale.

2.3. Copepod culture for feeding and respiration experiments

For determination of feeding and respiration, copepod *C. tenuiremis* were selected and transferred to 5 L beakers (with 5 L seawater) that was pre-equilibrated with ambient (390 μatm, LC) or elevated (1000 μatm, HC) CO_2 concentrations in a plant CO_2 chambers (HP1000G-D, Ruihua instrument & equipment Co. Ltd., China) and nursed for a period of 4 days. Each beaker held 300–500 individuals, while the sterilized seawater was renewed by 50% every 24 h and the diatom was added to the designed concentration (5×10^4 cells ml^{-1}), the temperature, light intensity and L:D cycle were the same as above. LC and HC cultures were continuously aerated (50 ml min^{-1}) so that the copepod was exposed to the same ambient O_2 level (outdoor) while exposed to the different levels of pH and pCO_2. The pH was measured as mentioned above and pCO_2 was controlled in the CO_2 chambers with variations less than 3%. Other parameters of the carbonate system were computed with CO2SYS software (Lewis and Wallace, 1998) based on the known values of pCO_2, pH, salinity (26) and nutrients (phosphate, 4 μmol L^{-1}; silicate, 40 μmol L^{-1}), the equilibrium constants K_1 and K_2 for carbonic acid dissociation after Roy et al. (1993), and K_B for boric acid after Dickson (1990) were used.

2.4. Determination of feeding rate

To measure the clearance and feeding rates, *C. tenuiremis* cultured under LC and HC condition for 24, 36 and 90 h were randomly selected and temporary raised in a 55 ml bottle (20 individuals) under the LC or HC conditions, with the diatom *P. tricornutum* at the same concentration as above. The diatom cells were counted with Z2 coulter (Beckman Coulter Counter, Buckinghamshire, UK). Bottles without copepod having the same diatom concentration were set as control. Stability of pH (pH variation less than 0.05) was achieved under both LC and HC conditions in the darkness at 20 °C during the 4 h feeding experiment. The clearance and feeding rates, obtained based on changes in the diatom cell concentration, were calculated according to Frost (1972).

2.5. Measurement of respiration rate

Respiration rates of *C. tenuiremis* were tested at time 0, 24, 48 and 72 h after nursed under LC and HC condition as mentioned above. The respiratory O_2 consumption was measured with a Clark type oxygen electrode (5300 A, YSI) which was calibrated each time before use, using outdoor-air aerated seawater as 100% O_2

saturation and the seawater deprived of O_2 with superfluous sodium sulfite (Na_2SO_3) (cross-checked with pure N_2-bubbled water) was used as 0%. The sterilized seawater was aerated with LC and HC air until oxygen reach saturation before used for the measurement of O_2 consumption. For the O_2 consumption determination, each pH level had four replicates, 30 individuals were randomly sampled and incubated in each bottle (55 ml) for 4 h (during which linear decrease in O_2 concentration was confirmed). During the measurement of respiration, no food organisms were provided. Changes in O_2 concentration was used to calculate respiration rate. The measurements were done in darkness at 20 °C without addition of the diatom.

2.6. Statistical analysis

Data were analyzed with *t*-test to establish differences among treatments, with a significant level set at $p = 0.05$.

3. Results

The carbonate system parameters of seawater were maintained stable during the periods for feeding rate or O_2 consumption measurements (Table 1). Compared to LC conditions, DIC, HCO_3^- and CO_2 significantly increased by 6.4% ($p = 0.006$), 9.5% ($p = 8.404 \times 10^{-5}$) and 147.7% ($p = 3.833 \times 10^{-13}$), while CO_3^{2-} decreased by 51.2% ($p = 1.943 \times 10^{-9}$) in the HC cultures, respectively (Table 1).

For behavioral sensitivity test, no difference was found in the distribution of the copepod individuals between the selective two areas through which the same pH seawater was run (Fig. 2). However, when the HC and LC seawaters were run simultaneously, significantly higher proportions of individuals were found at pH 8.15 area compared to pH 7.6 ($p = 0.002$) or pH 7.0 ($p = 7.400 \times 10^{-10}$) areas. However, no significant difference was found between pH 7.80 and pH 8.15 ($p = 0.750$).

Clearance and feeding rates decreased significantly by 31.4% ($p = 0.016$) and 31.6% ($p = 0.020$), respectively (Fig. 3B and C), and respiration rate, in the contrary, increased by 22.7% ($p = 0.010$) in 24 h after the exposure to the HC (pH 7.83) condition (Fig. 3A), reflecting decreased feeding with increased respiration at the initial of 24 h due to the chemical stressors associated with ocean acidification condition. However, with prolonged exposures, the HC-grown individuals increased the clearance and feeding rates (Fig. 3B and C), by 75.3% ($p = 0.054$) and 71.0% ($p = 0.057$) in 36 h and 40.9% ($p = 0.178$) and 50.3% ($p = 0.151$) in 90 h, respectively, compared to the LC condition (pH 8.18). The respiration rates were significantly increased by 28.7% ($p = 5.069 \times 10^{-4}$) and 35.4% ($p = 0.003$) in the HC-nursed individuals after 48 and 72 h (Fig. 3A), respectively.

4. Discussion

We found that the copepod could sense the high levels of CO_2 and associated increase of seawater acidity by avoiding the acidified areas. In nature, when confronted with adverse environments, mobile organisms usually show behavioral avoidance to cope with

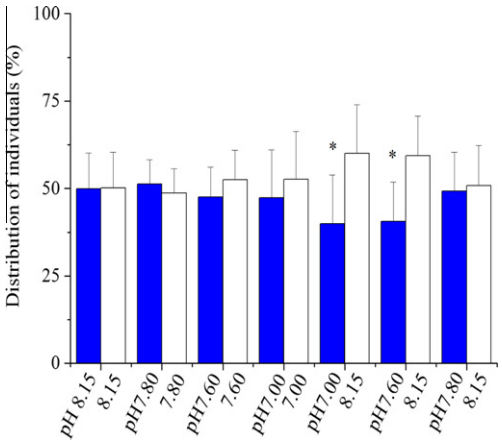

Fig. 2. The pH sensitivity of the copepod, *C. tenuiremis*. Distribution of *C. tenuiremis* individuals when gave choice to pH 8.15 versus pH 8.15, pH 7.8 versus pH 7.8, pH 7.6 versus pH 7.6, pH 7.0 versus pH 7.0, pH 7.0 versus pH 8.15, pH 7.6 versus pH 8.15 and pH 7.8 versus pH 8.15. The symbol "*" above the columns indicate significant ($p < 0.05$) differences between the two pH treatments. Values in the columns indicate the numbers of repeated experiments. Vertical bar means ± SD ($n = 8$–91).

disadvantageous conditions (Vetter and Smith, 2005). Ocean acidification may disorder olfactory or sensing capability for locating their ecological niche (Munday et al., 2009) or to avoid the predator (Dixson et al., 2010). Behavior changes caused by environment variety are known for a variety of marine animals, such as an avoidance reaction to anoxic pressure in a shrimp (Cook and Boyd, 1965; Haefner, 1971) and in benthic copepods (Tinson and Laybourn-Parry, 1985). In our study, copepod *C. tenuiremis* showed markedly avoidance behavior to lowered pH and preference to stay at the ambient level. The ability to perceive the chemical changes in seawater and to escape from the adverse circumstance is the key for *C. tenuiremis* to survive in coastal waters where pH changes are specially large especially when eutrophication and atmospheric CO_2 rise interacts (Cai et al., 2011).

To respond to and cope with the increased external acidity, the copepod *C. tenuiremis* increased its food acquisition to compensate for the extra energy demand via enhancement of respiration. Most organisms are more or less capable of maintaining the stability of intracellular acid–base to counteract external pH changes, using diverse ionic pumps and enzymes, such as H^+-ATPases and Na^+/K^+-ATPases (Pörtner et al., 2000), in addition, passive buffering and CO_2 exchanges also contribute to intracellular acid–base modulation (Whiteley, 2011), though the ability in such acid–base regulation is species-specific. Since acid–base regulation in the cells require additional energy when there is an acidic perturbation, the copepod *C. tenuiremis* under lowered pH condition, as shown in this study, grazed less at the initial phase but fed more with acclimation to meet the increased respiration and associated energy demand. In contrast, the jumbo squid *Dosidicus gigas* was found to down-regulate its metabolism rate under CO_2-induced acidity decrease (Rosa and Seibel, 2008), however, high tolerance to hypercapnic stress was also reported in a sipunculid worm

Table 1
Seawater carbon system parameters in the cultures. Data are the means ± SD of seven measurements. Parameters of the carbonate system were computed with CO2SYS software (Lewis and Wallace, 1998) based on the known values of pCO_2, pH, salinity, nutrients, the equilibrium constants K_1 and K_2 for carbonic acid dissociation after Roy et al. (1993), and K_B for boric acid after Dickson (1990).

	pH_{NBS}	DIC (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	CO_2 (μmol kg^{-1})	TA (μmol kg^{-1})
LC	8.18 ± 0.02*	1867.0 ± 77.5*	1705.8 ± 65.8*	147.8 ± 12.1*	13.4 ± 0.3*	2105.0 ± 91.9
HC	7.83 ± 0.02	1986.0 ± 46.2	1876.1 ± 41.2	72.2 ± 3.4	33.2 ± 1.6	2090.9 ± 45.0

* Indicates significant ($p < 0.05$) differences between the ambient and elevated CO_2 levels.

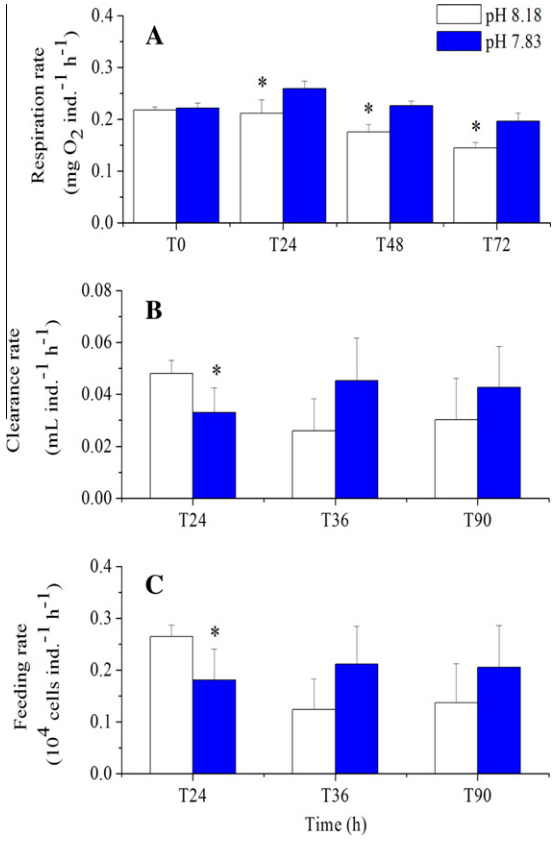

Fig. 3. Rates of respiration and feeding by *C. tenuiremis* under ambient (LC, 390 μatm, pH 8.18) and elevated (HC, 1000 μatm, pH 7.83) CO_2 concentrations. (A) Respiration, (B) clearance and (C) feeding rates of *C. tenuiremis*. The symbol "∗" above the columns indicate significant ($p < 0.05$) differences between the two pH treatments. Vertical bar means ± SD ($n = 3–4$).

Sipunculus nudus (Pörtner and Reipschläger, 1996). Increased ratio of CO_2 to O_2 can certainly influence the ventilation and respiration. The increased H^+ level in body compartments would down-regulate the oxygen affinity of respiratory pigments such as hemocyanin or hemachrome, which will have profound effects on ventilation (Taylor and Whiteley, 1989). In the present study, higher respiration rate found under the HC conditions coincided with increased demand for food in the copepod tested, reflecting an up-regulated metabolism. Second generation may respond differentially to ocean acidification compared to the first one (Dupont and Thorndyke, 2008), therefore, findings in the present study only reflect a short-term response of the copepod.

In a high CO_2 ocean, increased feeding pressure on the phytoplankton would be expected, though larvae of a sea urchin decreased their feeding rate in response to ocean acidification (Dupont and Thorndyke, 2008). Increased feeding pressure would influence species abundance and community structures of phytoplankton and fecal pellets excreted by zooplankton are expected to increase with the ongoing increase of atmospheric CO_2 concentration. In view of the marine biological CO_2 pump, carbon transport to deep oceans will be likely increased provided that both photosynthetic carbon fixation by phytoplankton and fecal pellets produced by zooplankton increase to respond to ocean acidification.

Author contributions

K.S.G. contributed to the design of the study, W.L. contributed in the experiment implementation, W.L. and K.S.G. analyzed and interpreted the data and drafted the paper.

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