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The larvae of congeneric gastropods showed differential responses to the combined effects of ocean acidification, temperature and salinity

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ABSTRACT

The tolerance and physiological responses of the larvae of two congeneric gastropods, the intertidal *Nassarius festivus* and subtidal *Nassarius conoidalis*, to the combined effects of ocean acidification (pCO_2 at 380, 950, 1250 ppm), temperature (15, 30 °C) and salinity (10, 30 psu) were compared. Results of three-way ANOVA on cumulative mortality after 72-h exposure showed significant interactive effects in which mortality increased with pCO_2 and temperature, but reduced at higher salinity for both species, with higher mortality being obtained for *N. conoidalis*. Similarly, respiration rate of the larvae increased with temperature and pCO_2 level for both species, with a larger percentage increase for *N. conoidalis*. Larval swimming speed increased with temperature and salinity for both species whereas higher pCO_2 reduced swimming speed in *N. conoidalis* but not *N. festivus*. The present findings indicated that subtidal congeneric species are more sensitive than their intertidal counterparts to the combined effects of these stressors.

1. Introduction

Ocean acidification (OA) is one consequence of the ever-increasing concentration of atmospheric carbon dioxide driven by anthropogenic releases since the Industrial Revolution. With such carbon dioxide continually dissolving in sea water by air–sea fluxes, global surface ocean pH is projected to decrease by 0.3–0.4 units within this century and 0.7 units after 300 years (Caldeira and Wickett, 2003). Such an alarming decrease may have significant effects on the physiology, biochemistry and evolutionary future of many marine organisms, altering ecological balance and biodiversity (Turley et al., 2011). For example, the northern rock barnacle *Semibalanus balanoides* showed a decrease in survival after exposure to pH 7.7 seawater (Findlay et al., 2009), while Kurihara (2008) found that egg production in the Indian bait prawn *Palaemon pacificus* was reduced after 15 weeks of exposure to pH 7.6 seawater. Many recent studies have thus suggested that the consequence of OA could be profound in affecting the biology of many organisms in the marine ecosystems (e.g., Landes and Zimmer, 2012; Li and Gao, 2012).

Early life stages are considered to be more sensitive to environmental stress than maturity stages and its effects have received much attention (reviewed by Dupont and Thorndyke, 2009), with crucial implications for population abundances, species diversity and ecosystem functions. Calcifying larvae of molluscs uniformly exhibit a decrease in shell growth under elevated pCO_2 levels (Bechmann et al., 2011). Developmental time was extended by low seawater pH and larval size was smaller for the green sea urchin *Strongylocentrotus droebachiensis* (Dupont et al., 2013). Larvae of the Japanese rice fish *Oryzias latipes* were also more sensitive than adults to reduced pH and showed a decrease in gene expression and metabolic rate (Tseng et al., 2013). On the other hand, other species may benefit from OA. For instance, the common sun sea star *Crossaster papposus* showed an increase in growth upon exposure to OA stress (Dupont et al., 2010).

Acidification does not occur in isolation, but in concert with a complex of related stressors, such as global warming, eutrophication and increased UV radiation. Few studies, however, have examined the resultant interactions, which may aggravate the effect of OA on marine organisms (Hendriks et al., 2010). Nevertheless, the combined effects of acidification and elevated temperature have been reported for bryozoans (Rodolfo-Metalpa et al., 2010), barnacles (Findlay et al., 2010), corals (Anthony et al., 2008), limpets (Findlay et al., 2008), sea urchins (Byrne et al., 2010) and fish (Munday et al., 2009), and acidification and reduced salinity have been studied in amphipods (Egilsdottir et al., 2009).

Intertidal or shallow-water species are expected to develop a higher tolerance for greater fluctuations in environmental changes, including OA. For example, the shallow-water Dungeness crab *Cancer magister* can regulate extracellular acid–base balance during

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short-term hypercapnia but not for the deep-sea Tanner crab *Chionoecetes tanneri* (Pane and Barry, 2007). Species-specific sources of heterogeneity constitute one of the major biological factors that explain variations in responses to OA (Kroeker et al., 2010). However, inter-specific differences may be environmentally driven (Kurihara et al., 2004). To reduce the bias due to taxonomic differences, a better approach would be to compare the responses of congeneric species from contrasting habitats.

Family Nassariidae is one of the largest families in Class Gastropoda with many members being saprophagous (Cernohorsky, 1984). *Nassarius festivus*, a nassariid gastropod dominating intertidal sandflats in Hong Kong, plays an important role in energy recycling by consuming large amounts of carrion on the shore (Morton, 1990). This species was found to be tolerant to starvation, low salinity and hypoxia (Cheung, 1997; Cheung and Lam, 1999; Cheung et al., 2008). Its congeneric counterpart *Nassarius conoidalis* is a subtidal scavenger dominating southern waters in Hong Kong and its physiological responses to hypoxia, temperature and salinity have been well-documented (Zhao et al., 2009; Liu et al., 2011).

In this present study, we investigated the mortality, swimming behavior and respiration rate of the veliger larvae of *N. festivus* and *N. conoidalis* under the combined effects of varying levels of pCO_2, temperature and salinity. As it lives in subtidal habitats with smaller fluctuations in environmental factors, we hypothesized that *N. conoidalis* would be more sensitive and much affected by these stressors compared with its intertidal counterpart.

2. Materials and methods

2.1. Study organisms

Adult *N. festivus* (shell length: 13 ± 2 mm) were collected from a sandy shore at Starfish Bay, Hong Kong (22.48°N, 114.24°E), while adult *N. conoidalis* (shell length: 22 ± 2 mm) were trawled from southern Hong Kong waters (22.17°N, 114.17°E) at a depth of 10–30 m in November 2011. The two species were maintained separately in seawater at a temperature of 24 °C and salinity of 30 psu, which are the average ambient conditions for these gastropods in their natural habitats (Liu et al., 2011). Individuals were fed twice a week with the short-necked clam *Ruditapes philippinarum* according to the feeding frequency of the animals observed in the field (Cheung, 1994). Seawater was changed immediately after feeding for 2 h to avoid the accumulation of metabolic wastes (Cheung et al., 2008; Liu et al., 2011). Egg capsules spawned on the walls of aquaria were transferred to chambers containing 0.45 μm-filtered seawater and cultured at 30 °C and 30 psu in order to maintain a stock of competent larvae for subsequent experiments. Larvae hatched within 24 h were used in all treatments.

2.2. Experimental design

The combined effects of pCO_2, temperature and salinity on the larvae were studied using a full factorial experiment with three levels of pCO_2, two levels of temperature and two levels of salinity. pCO_2 levels of 380 ppm (LC), 950 ppm (MC), and 1250 ppm (HC) constitute the present-day situation and scenarios for 2100 and 2300 respectively, as projected by the report of Intergovernmental Panel on Climate Change (IPCC, 2007). 15 °C (LT) and 30 °C (HT) represented the median temperature in winter and summer in Hong Kong waters (EPD, 2012). A chiller was used to maintain the seawater at 15 °C and a thermostatically controlled circulator was used to keep the seawater at 30 °C. 10 psu (LS) represented the lowest salinity the gastropods would be exposed to in summer due to the freshwater input from the Pearl River of mainland China (Zong et al., 2010), whereas 30 psu (HS) is the normal salinity of Hong Kong seawater (EPD, 2012). Various CO_2 partial pressures were prepared according to Findlay et al. (2008). Air and industrial CO_2 gas (purity of 99.5%, Hong Kong Oxygen & Acetylene Co. Ltd.) were mixed well by regulating their flow rates using digital flow meters (GCR-B9SA-BA15, Vogtlin, Sweden) in sealed bottles containing water and dried in conical flasks before being bubbled into different treatments. Only air was bubbled for the control group with ambient CO_2 level. Temperature, pH, pCO_2 and salinity were recorded every day. A carbon dioxide online analyzer (LI-COR, LI-820, USA) was used to measure the real time pCO_2 level. Software CO2SYS (Lewis and Wallace, 1998) was used to calculate the saturation of calcite (ΩCa) and aragonite (ΩAr), total alkalinity (At) and the relationship between these parameters. Total alkalinity was also checked by an alkalinity titrator (HANNA, HI 84431, Germany) every week. Table 1 shows the environmental parameters of the 12 treatments.

2.3. Larval mortality

Thirty-six bottles with 60 larvae in each bottle were assigned to 12 treatments with 3 replicates for each treatment. Larvae hatched within 24 h were immediately transferred into glass bottles at a density of approximately 1 larva per 2 ml seawater. Filtered seawater (HA Millipore filter, 0.45 μm pore size) with antibiotics (penicillin G sodium salt and streptomycin sulfate (Sigma, Germany)) added at a concentration of 50 μg ml^{-1} (Strathmann, 1987) was renewed every 24 h, adjusted to desired temperatures, salinities and pCO_2 levels beforehand. Cumulative mortality was recorded at 24 h, 48 h and 72 h. Larvae with no velar lobe ciliary movement were considered dead and removed immediately when encountered (Chan et al., 2008). The diatom *Thalassiosira pseudonana* was provided as food at a concentration of 20×10^4 cells ml^{-1}.

Table 1
Environmental parameters of the 12 treatments (mean ± SD) (L: low, M: middle, H: high; T: temperature, C: pCO_2, S: salinity).

	Temperature (°C)	Salinity (psu)	pCO_2 (ppm)	pH	At (mg l^{-1})	ΩCa	ΩAr
LT–LC–LS	15.2 ± 0.2	10.1 ± 0.4	366 ± 27	7.80 ± 0.18	90.3 ± 15.4	0.58	0.33
LT–LC–HS	15.5 ± 0.3	30.0 ± 0.7	366 ± 27	8.05 ± 0.09	199.3 ± 11.9	4.62	2.93
LT–MC–LS	15.4 ± 0.1	10.1 ± 0.3	865 ± 83	7.50 ± 0.07	97.7 ± 0.6	0.33	0.19
LT–MC–HS	15.2 ± 0.1	30.3 ± 0.2	865 ± 83	7.75 ± 0.02	201.1 ± 19.4	2.14	1.36
LT–HC–LS	15.3 ± 0.1	10.3 ± 0.4	1260 ± 147	7.34 ± 0.09	97.3 ± 5.5	0.21	0.12
LT–HC–HS	15.5 ± 0.3	29.8 ± 0.4	1260 ± 147	7.52 ± 0.04	197.9 ± 6.1	0.98	0.62
HT–LC–LS	29.1 ± 0.7	10.7 ± 0.8	366 ± 27	7.84 ± 0.10	87.3 ± 17.2	1.20	0.71
HT–LC–HS	29.4 ± 0.4	31.0 ± 0.7	366 ± 27	8.18 ± 0.08	199.0 ± 10.8	7.73	5.11
HT–MC–LS	29.2 ± 0.6	10.8 ± 0.5	865 ± 83	7.56 ± 0.05	102.8 ± 4.2	0.75	0.44
HT–MC–HS	29.4 ± 0.3	30.3 ± 0.6	865 ± 83	7.70 ± 0.07	198.1 ± 8.8	3.18	2.10
HT–HC–LS	29.3 ± 0.6	11.0 ± 0.4	1260 ± 147	7.37 ± 0.06	96.9 ± 2.1	0.41	0.24
HT–HC–HS	29.2 ± 0.6	30.5 ± 0.5	1260 ± 147	7.57 ± 0.06	199.5 ± 3.9	1.91	1.26