ISJ 15: 14-18, 2018 ISSN 1824-307X

SHORT COMMUNICATION

Effects of water temperature and pH value on covering behavior of the sea urchin *Glyptocidaris crenularis*

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Accepted November 28, 2017

Abstract

As marine calcifying organisms, sea urchins are sensitive in the changing ocean. More information is needed about the effects of pH value and water temperature on behaviors of sea urchins. Here, we reported that pH value and water temperature significantly affected the covering behavior of sea urchins *Glyptocidaris crenularis*. Lower pH value (pH = 7.4) significantly reduced the time to first covering (p = 0.026), while significantly decreased number of covered sea urchins (p = 0.029) and number of shells used for covering (p = 0.007) in *G. crenularis*. Water temperature did not significantly affect the time to first covering (p = 0.180) or number of covered sea urchins (p = 0.157), though significantly affected number of shells used for covering (p = 0.042). The present study provides preliminary information on behavioral ecology of sea urchins. Notably, the effects of CO_2 induced acidification should be further investigated in future.

Key Words: sea urchin; covering behavior; pH value; water temperature

Introduction

Over the past 250 years, Atmospheric carbon dioxide (CO_2) levels increased by approximately 40 % (Solomon *et al.*, 2007), one third of which has been taken up by the oceans (Sabine *et al.*, 2004). Due to absorbing increasing amounts of CO_2, ocean warming and acidification have been identified as the greatest anthropogenic threat to marine ecosystems (Halpern *et al.*, 2008; de Madron *et al.*, 2011). This highlights the risks to marine calcifying organisms in future (Orr *et al.,* 2005). As marine calcifying organisms, sea urchins are sensitive in the changing ocean (Dupont *et al.,* 2013). Ocean warming and acidification have showed greatly impacts on reproduction (Reuter *et al.,* 2011), gamete health (Morgan and Galione, 2007), larval development (Brennand *et al.*, 2010; Stumpp *et al.*, 2011a, b) calcification (Byrne *et al.,* 2011) and gene expression (O'Donnell *et al.,* 2010) of sea urchins. Effects of pH value and water temperature on behaviors of sea urchins remain largely unknown, although the fragility has showed in various of behaviors of marine fish (Munday *et al.,* 2009; Cripps *et al.*, 2011; Simpson *et al.,* 2011) and bivalves (Chan *et al.,* 2011).

Covering behavior refers to echinoids using their tube feet and spines to manipulate environmental objects, such as shells, stones and algae fragments, to put onto their dorsal surface (Verling *et al.*, 2002). Covering behavior has been well proposed to have multiple functions of protection from lights (Verling *et al.,* 2002), predation (Agatsuma, 2001), floating sand (Richner and Milinski, 2000) and wave surge (Dumont *et al.,* 2007). Recently, effects of elevated temperature and acidification have been investigated separately (Challener and McClintock, 2013; Zhao *et al.*, 2014; Brothers and McClintock, 2015; Zhang *et al.*, 2017). The effects of water temperature and pH value on covering behavior, however, were not simultaneously investigated.

Acidification can be experimentally imitated by CO_2 (for example, Dupont *et al.*, 2013) or HCl (for example, Yamada and Ikeda, 1999) in laboratory. A comparative study indicates that HCl-induced acidification showed lower acute toxicity to aquatic animals than that induced by CO_2 (Zhang *et al.*, 2011).

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Table 1 Characteristics of sea urchins and covering materials

	Water temperature	15°C		25°C	
	pH value	7.4	8.2	7.4	8.2
Glyptocidaris crenularis	Test diameter (mm)	16.55 ± 1.98	16.44 ± 1.76	16.58 ± 2.04	16.50 ± 1.89
	Test height (mm)	7.87 ± 1.14	8.20 ± 0.98	8.09 ± 0.98	8.16 ± 1.23
	Body weight (g)	2.04 ± 0.73	2.10 ± 0.71	2.10 ± 0.74	2.06 ± 0.74
Mytilus galloprovincialis	Shell length (mm)	11.82 ± 0.81	11.91 ± 0.85	11.74 ± 0.84	11.59 ± 0.76
	Shell height (mm)	19.84 ± 2.61	20.31 ± 1.45	19.96 ± 1.39	19.45 ± 2.02
	Shell weight (g)	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.12 ± 0.02

(Mean ± SD, N = 36 for *Glyptocidaris crenularis*, N = 40 for *Mytilus galloprovincialis*)

Thus, HCl-induced acidification can be used to provide preliminary information on behavioral response of sea urchins to ocean acidification.

The aim of the present study is to investigate the effects of water temperature and pH value on the covering behavior of the sea urchin *Glyptocidaris crenularis*.

Materials and Methods

Sea urchins

Glyptocidaris crenularis were originally reared at Dalian Haibao Seafood Company in Lvshun of Dalian, China. Individuals from one cohort were transported to Key Laboratory of North Mariculture and Stock Enhancement, Dalian Ocean University, China. All sea urchins were acclimated for 7 days at 19 - 21 °C of water temperature, 30 - 31 ‰ of salinity and 8.2 of pH before the behavioral experiment. There was no significant difference of test diameter, test height and body weight among experimental groups in *G. crenularis* ($p > 0.05$, Table 1).

Experimental design

Geochemical models indicates that ocean acidification will be over 1.4 pH units in the next 300 years (Caldeira and Wickett, 2003). Consequently, two pH values (7.4 and 8.2) were comparatively studied in the coral *Oculina patagonica* (Fine and Tchernov, 2007). We accordingly set pH = 7.4 and 8.2 in the present study. The low pH value (pH = 7.4) was simply produced by adding hydrochloric acid to the natural seawater (pH = 8.2). To maintain water temperatures, the behavioral experiments were carried out in buckets (diameter of the bucket bottom = 22 cm) bathed in temperature-controlled tanks, where two water temperatures (15 °C and 25 °C) were set. Forty shells of *Mytilus galloprovincialis* were randomly selected and put into each bucket according to our previous study (Zhao *et al.*, 2014). There were no significant differences in shell length, shell width and body weight of *M. galloprovincialis* among experimental groups (Table 1, $p > 0.05$). We then placed 144 sea urchins into the 12 buckets (12 individuals per bucket, 4 treatments, 3 replicates). Because the experimental duration was relatively short, pH values were well maintained. Time to first covering refers to the time of covering by the first sea urchin that covered, indicating the behavioral reaction. The number of covered sea urchins and number of shells used for covering were measured every 10 min during 90 min, indicating the behavioral capability.

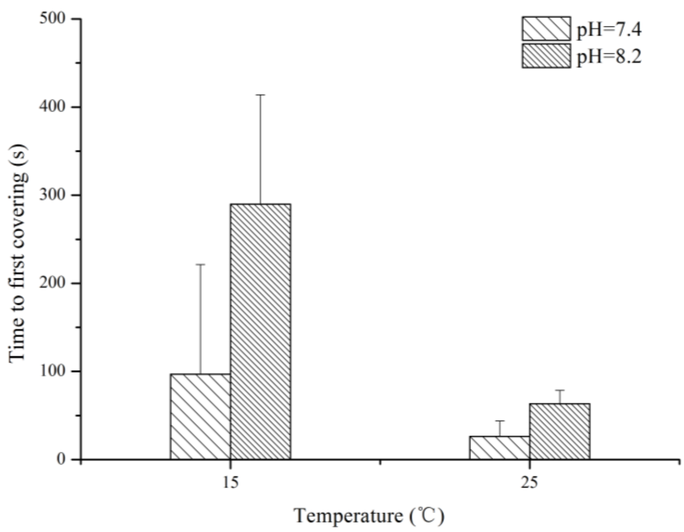

Fig. 1 Time to first covering of *Glyptocidaris crenularis* at different water temperatures and pH values (N = 3, mean ± SD).

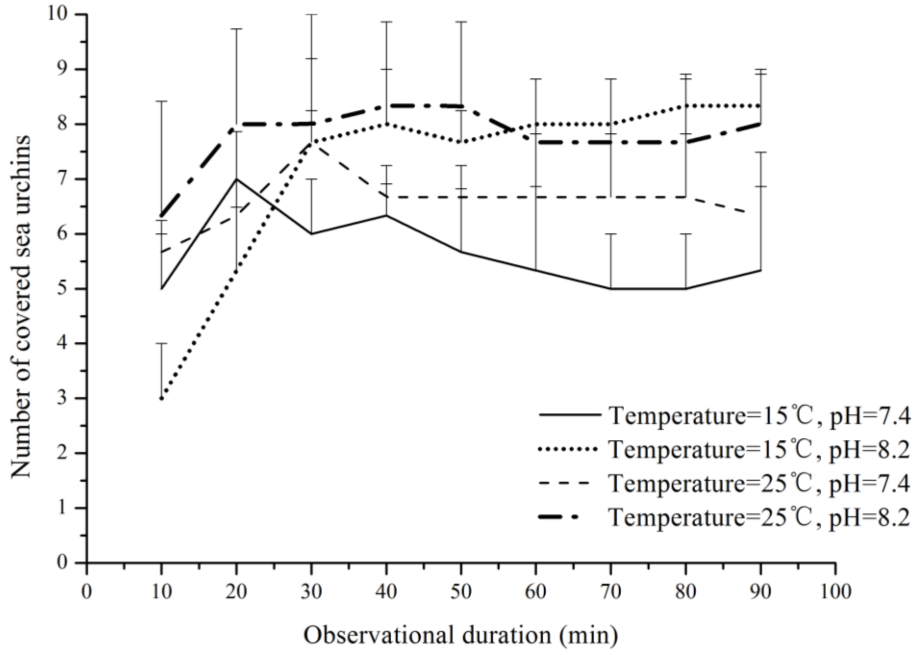

Fig. 2 Number of covered *Glyptocidaris crenularis* at different water temperatures and pH values (N = 3, mean ± SD).

Statistical analysis

All original data were tested for normal distribution and homogeneity of variance. Repeated measured ANOVA was used to detect the differences of number of covered sea urchins and number of shells used for covering among experimental groups. Time to first covering was analyzed using Mann-Whiney U test because the data showed heterogeneity of variance. All analysis was carried out using SPSS 13.0. A probability level of $p < 0.05$ was considered statistically significant.

Results

Time to first covering

Time to first covering was significantly lower in sea urchins exposed to lower pH ($p = 0.026$, Fig. 1). However, water temperature did not significantly affect time to first covering, although *G. crenularis* showed obviously quicker reaction at 25 °C than those at 15 °C ($p = 0.180$, Fig. 1).

Number of covered sea urchins and number of shells used for covering

Lower pH significantly decreased number of covered sea urchins ($p = 0.029$, Fig. 2) and number of shells used for covering ($p = 0.007$, Fig. 3). Water temperature did not significantly affect number of covered sea urchins ($p = 0.157$, Fig. 2), though significantly affected number of shells used for covering ($p = 0.042$, Fig. 3). Interaction between water temperature and pH value showed no significant effect on number of covered sea urchins ($p = 0.752$) or number of shells used for covering ($p = 0.989$). Observational duration significantly affected both number of covered sea urchins ($p < 0.001$) and number of shells used for covering ($p = 0.01$). Both number of covered sea urchins and number of shells used for covering increased in the first 20 or 30 min. and then obviously decreased in lower pH groups (Figs 2, 3).

Discussion

Water temperature and pH value significantly affected calcification (Byrne *et al.,* 2011), growth (Albright *et al.,* 2012) and behavior (Cripps *et al.,* 2011) of marine organisms, ultimately impacting the entire ecosystem (Strandberg *et al.,* 2012). Considering their roles in structuring marine benthic communities, it is critical to understand ecological response of sea urchins to water temperature and pH value. The present study investigated the behavioral response of sea urchins to water temperatures and pH values. It provides preliminary information on behavioral ecology of sea urchins.

In the present study, we found that lower pH value (pH = 7.4) significantly affected covering behavior of *G. crenularis*. This result is in agreement with previous reports on the behavioral response of marine organisms to pH values. In reef fish *Pseudochromis fuscus*, for example, ocean acidification significantly disturbed its prey detection (Cripps *et al.,* 2011). Lower pH value significantly decreased the number of covered sea urchins and number of shells used for covering. This result is not in agreement with the finding of Challener and McClintock (2013) that ocean acidification did not significantly impact covering behavior of juvenile sea

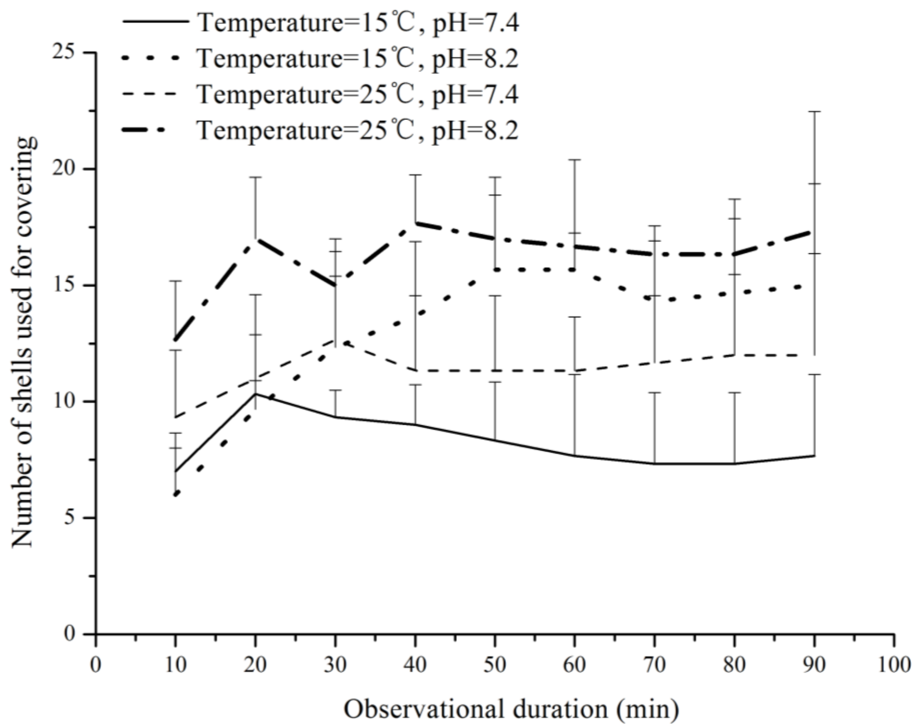

Fig. 3 Number of shells used for covering of *Glyptocidaris crenularis* at different water temperatures and pH values (N = 3, mean ± SD).

urchins *Lytechinus variegatus*. The disagreement is probably due to the difference of behavioral response to ocean acidification between the two species. Interestingly, we found that lower pH value significantly increased the speed of covering reaction. A reasonable explanation is that the acute stress increased the covering reaction. Consistently, Brothers and McClintock (2015) found that acute exposure to elevated temperature significantly increased covering behavior of *L. variegatus*, compared to chronical exposure.

Water temperature did not significantly affect the time to first covering and number of covered sea urchins. Crook (2003) found that *Paracentrotus lividus* covered more quickly during summer time and obviously less in winter time. These results confirm the difference of the covering behavior among different species (Verling *et al.,* 2004). Interestingly, we found number of shells used for covering was significantly different between sea urchins at 15 °C and 25 °C, although the *p* value was very close to 0.05 (p = 0.042). This result is not consistent with our previous study, in which we found no significant difference of number of shells used for covering between *G. crenularis* in 15 °C and 25 °C (Zhao *et al.*, 2014). This disagreement might be due to limited sample size in both studies and strongly highlights the individual variation of covering behavior of sea urchins.

Considering the multiple functions of covering behavior, our finding indicates that lower pH value could probably trigger a series of indirect ecological impacts on sea urchins (at least on *G. crenularis*). Notably, the present study used HCl rather than CO_2 to set the pH value. The effects of CO_2 induced acidification should be further investigated.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (41506177) and the Chinese National 863 Project (2012AA10A412). We thank H Zhou, X Tian and W Feng for their assistance. All authors have no conflict of interest.

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