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Boldness in a Deep Sea Hermit Crab to Simulated Tactile Predator Attacks is Unaffected by Ocean Acidification

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Abstract – Despite rapidly growing interest in the effects of ocean acidification on marine animals, the ability of deep-sea animals to acclimate or adapt to reduced pH conditions has received little attention. Deep-sea species are generally thought to be less tolerant of environmental variation than shallow-living species because they inhabit relatively stable conditions for nearly all environmental parameters. To explore whether deep-sea hermit crabs (*Pagurus tanneri*) can acclimate to ocean acidification over several weeks, we compared behavioral "boldness," measured as time taken to re-emerge from shells after a simulated predatory attack by a toy octopus, under ambient (pH ~7.6) and expected future (pH ~7.1) conditions. The boldness measure for crab behavioral responses did not differ between different pH treatments, suggesting that future deep-sea acidification would not influence anti-predatory behavior. However, we did not examine the effects of olfactory cues released by predators that may affect hermit crab behavior and could be influenced by changes in the ocean carbonate system driven by increasing CO_2 levels.

Key words – deep sea, behavior, boldness, carbon dioxide, hermit crab, ocean acidification

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1. Introduction

Future ocean pH is expected to decrease noticeably at all depths as surface water continues to absorb rising amounts of atmospheric CO_2 (Ilyina et al. 2010; Ilyina and Zeebe 2012). The phenomenon of ocean acidification is a growing concern for the stability and sustainability of marine ecosystems (Fabry et al. 2008; Doney et al. 2009). Due to the accumulation of respiratory CO_2, the pH of deep ocean waters is naturally lower than surface waters (Feely et al. 2008). According to the SRES A1B scenario, deep sea (ca. 1000 m) pH is projected to decrease by 0.2–0.4 units by the end of this century (Ilyina et al. 2010), and under the RCP 8.5 scenario, with larger CO_2 emissions, bathyal pH could decrease by over 0.4 units (Gattuso et al. 2014). Increased CO_2 and associated carbonate chemistry changes in the deep sea could influence physiological processes contributing to the individual performance and population viability of deep-sea animals. Some studies indicate that deep-sea animals are less tolerant of pH changes than surface-water species are, due to the environmental stability (oxygen, temperature, pH, etc.) of deep-sea waters (Pane and Barry 2007; Taylor et al. 2014).

High environmental CO_2 levels can have negative impacts on the physiology and behavior of marine animals (Briffa et al. 2012; Clements and Hunt 2015). Increased CO_2 levels can modify metabolic rates of organisms (Bibby et al. 2007; Wood et al. 2008; Lee and Kim 2016), deteriorate olfactory functions of animals (de la Haye et al. 2012; Nilsson et al. 2012), deter homing ability (Munday et al. 2009), predator/prey detection (Munday et al. 2009; Dixson et al. 2010; Cripps et al. 2011), and resource assessment and decision making (de la Haye et al. 2011). Exposure to low seawater pH can also alter defensive behaviors in prey species, perhaps rendering them more vulnerable to potential predators (Bibby et al. 2007). Behavioral changes arise from various factors, ranging from developmental and physiological costs, to the impairment of the ability to gather and assess information and to make decisions, or avoidance of localized pollution (Briffa et al. 2012).

Here we investigated the influence of CO_2-driven reduction in

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*Corresponding author. E-mail: ktwon@kiost.ac.kr pH on anti-predatory behavior of the deep-sea hermit crab *Pagurus tanneri* (J. E. Benedict, 1892). In a previous study on this species, exposure of hermit crabs to reduced pH seawater led to a reduction in their antennular flicking behavior and a reduced ability to detect prey (Kim et al. 2016). To determine if the anti-predatory behavior of *P. tanneri* is affected by ocean acidification, we tested the hypothesis that the boldness of *P. tanneri* after simulated predator attack is altered by acidification. Upon disturbance or attack, hermit crabs withdraw into their host shell for a period, then emerge to resume activity. Although variation among individuals has been observed (Briffa and Twyman 2011), there is an optimal duration for seclusion that balances the trade-off between predation risk *vs.* feeding or mating behavior (Kim et al. 2009). We measured boldness as the time (seconds) for *P. tanneri* to emerge following simulated predator tactile attack.

2. Materials and Methods

Collection and maintenance of *P. tanneri*

Hermit crabs were collected at 884 m depth (36.71°N 122.28°W) using a suction sampler of the Remotely Operated Vehicle (ROV) *Doc Ricketts* (Dive DR306) on Oct 22nd, 2011. A total of 32 individuals were collected and transferred to an aquarium with seawater at *in situ* temperature (5°C) and 100% saturated with oxygen, at pH 8.0. *P. tanneri* tolerates these conditions for at least several months without any indication of stress (Kim et al. 2016). On Nov 30th, 2011, approx. 1 month after collection, 30 crabs were selected and all of the crabs were fed to satiation with chopped squid. Each crab was assigned to a 1 L transparent glass jar, coded, and assigned randomly to one of two pH treatment groups. Chilled (5°C) ambient (100% saturated with O_2, pH 8.0, total scale) seawater was supplied to each jar by tubing from a gas-controlled, flow-through aquarium system. Thirty jars (each housing 1 crab) were divided among 10 small (10 L) aquaria overflowing with treatment water. Water was delivered at 60 ml/min to each jar from the treatment reservoir through a 3 cm diameter PVC manifold, and a 10 mm diameter hose. On the next day, claw size and shell length of each crab were measured using digital calipers. There were no significant differences in the larger claw length (Mann-Whitney *U* test, $U = 111, P = 0.9504$) or shell length ($U = 108, P = 0.8519$) between pH treatment groups. Crabs were maintained in a darkened room with only dim red light, from the time of crab placement in jars until the end of the experiment for at least 4 weeks.

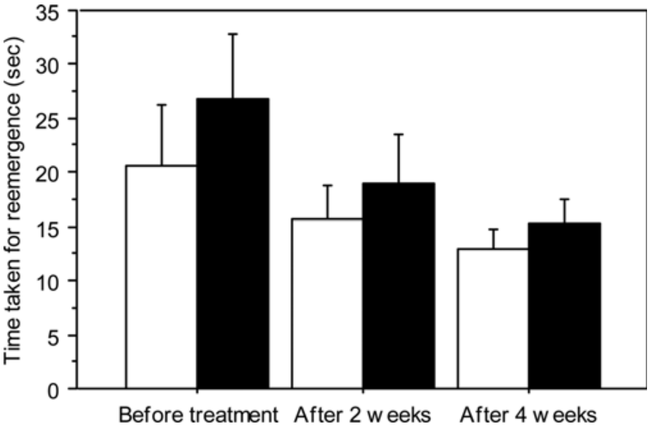

Fig. 1. *Pagurus tanneri*. Time taken for re-emergence (Mean ± s.e.) of hermit crabs after predatory attacks under low pH (pH 7.1, black bar) and control (pH 7.6, white bar) conditions

Seawater chemistry

Experimental conditions for seawater pH, dissolved oxygen, and temperature were maintained using a gas-controlled aquarium system (Barry 2008). Oxygen (Aanderaa Inc., model 3835, www.aadi.no), pH (Honeywell DuraFET III) and temperature were logged continuously (1 Hz) using a LabVIEW (National Instruments Corp.) application. Control of seawater pH is achieved via a PID feedback algorithm integrated with CO_2, N_2, and oxygen mass flow controllers (MFC, Sierra Instruments, Inc.) to maintain setpoints for oxygen and pH in seawater reservoirs that supply experimental treatment waters.

From Dec 1 to Dec 5, 2011, seawater pH delivered to both treatment groups was gradually adjusted from pH 8.0 to pH 7.6 (0.1 unit/day), and the dissolved oxygen (DO) level was simultaneously changed from 300 μM to 30 μM. pH 7.6 and DO 30 μM are *in situ* seawater values at their depth of collection (Barry, unpublished data). The pH of group one (low pH treatment) was then gradually adjusted from pH 7.6 to pH 7.1 (0.1 unit/day) between Dec 19 and Dec 21, 2011. A difference of 0.5 pH units was maintained between the low-pH (pH 7.1) and control (pH 7.6) treatments throughout the experiment (Table 1). Temperature and DO showed no differences across treatments (Table 1). Periodic measurements of pH using a spectrophotometric pH method (Byrne et al. 1999) (Low pH: 7.11 ± 0.01, Control pH: 7.58 ± 0.04) showed only negligible difference from measurements performed using the Honey Well Dura FET pH sensors (Table 1). Seawater DO in each jar was also measured periodically during the experiment using Aanderraa® oxygen optodes (model 3835) to ensure

Table 1. Carbonate system and other physical parameters for experimental treatments measuring the response of hermit crabs (Mean ± SD)

	Low pH	Control
pH	7.12 (± 0.02)	7.6 (± 0.01)
TCO_2	2365.57 ± 36.68	2677.67 ± 270.78
Salinity (ppt)	33.0 ± 0.1	33.0 ± 0.1
Temperature (^oC)	6.0 ± 0.1	6.0 ± 0.1
PCO_2 (μatm)	3596.47 ± 159.18	1378.82 ± 111.55
Alkalinity (μEq/kg)	2207.86 ± 36.19	2687.47 ± 274.02
Calcite saturation	0.36 ± 0.02	1.31 ± 0.18
Aragonite saturation	0.23 ± 0.01	0.83 ± 0.12
HCO_3^- ($\mu mole \cdot kg^{-1}$)	21167.51 ± 35.09	2553.16 ± 258.80
CO_3^{2-} ($\mu mole \cdot kg^{-1}$)	15.00 ± 0.81	54.32 ± 7.60

The parameters were calculated with CO2sys (Pierrot et al. 2006) using the pH and TCO_2 values with dissociation constants from Dickson and Millero (1987) and KSO_4 using Dickson (1990)

the expected DO was maintained. To determine the calcite and aragonite saturation states of treatment waters, samples were collected from all treatments 5 times and DIC (Dissolved Inorganic Carbon) was measured by non-dispersive infrared analysis (LI-COR model 6262), as detailed by Friederich et al. (2002). The parameters except pH, TCO_2, salinity, and temperatures in Table 1 were calculated with CO2sys (Pierrot et al. 2006) using the pH and TCO_2 values with dissociation constants from Dickson and Millero (1987) and KSO_4 using Dickson (1990).

Boldness (emergence time after attack)

Boldness for each crab in both experimental groups was measured in individual trials. Each crab was carefully transferred from its jar to a 10 L aquarium (15 × 30 × 20 cm) filled with the appropriate treatment or control seawater and left undisturbed for 5 minutes to adjust to its new surroundings. The crab was then overturned using a toy rubber octopus (20 cm in length) mimicking an attack by a potential predator to prompt it to retreat into its shell (ESM 2). The attack ceased when the hermit crab hid in the shell completely. Once the attack was terminated, the time until the crab emerged to stand on the aquarium bottom was measured using a stopwatch. Boldness measurements of the individual crabs were performed repeatedly before exposure to different pH treatments, 2 weeks and 4 weeks after pH stabilized following the pH adjustment period. Measurement for five crabs could not be done repeatedly because two crabs in the control group were dead at 2 weeks after exposure and 3 additional crabs (1 in control and 2 in low pH treatment) were dead at 4 weeks after exposure.

All live crabs were tested 3 times on the same day at each of the three sampling points. The time interval between trials for each crab was 1–2 min.

Statistical analysis

Boldness values for each crab on the same day were averaged. To determine if the responses are different among aquaria, we conducted an ANOVA for each measurement period in each pH treatment. Because there was no significant effect of aquaria on the response, we regarded each crab in each jar as an independent replicate ($N = 15$). Because there were some missing values in repeated measures, a Linear Mixed Model (LMM) was used to test the effect of pH treatment, Time and the pH × Time interaction on the boldness behavior of the crabs. Based on AIC (Akaike's Information Criterion), we chose a heterogeneous compound symmetry model as a covariance structure. To determine if the variances of the differences in boldness between all possible pairs of groups are equal, we conducted the Mauchly's Test of Sphericity. There was no violation of Sphericity assumption ($p > 0.05$). SPSS 13.0 for Windows was used for this analysis.

3. Results

Boldness responses among hermit crabs did not differ between pH treatments before ($F_{1,27} = 1.179, P = 0.287$) or after ($F_{1,26.348} = 1.118, P = 0.300$) exposure to reduced pH seawater. Nor was there a significant effect of exposure time (LMM: effect of time, i.e. 2 and 4 weeks after exposure; $F_{1,26.272} = 3.023$, $P = 0.094$), or interaction between pH treatment and Time ($F_{1,26.272} = 0.140, P = 0.686$).

4. Discussion

The result that the boldness response of *P. tanneri* following a simulated attack was not influenced by lower pH conditions suggests that near future levels of CO_2 would not influence decision-making related to at least some aspects of predator recognition and risk assessment. *P. tanneri* did not show instantaneous hiding behavior in response to simulated predator attacks, as is observed in shallow water or land hermit crabs (Briffa and Twyman 2011). Because the boldness response varies greatly among individual hermit crabs under normal conditions (Briffa et al. 2008b), significant change in this behavioral response to an increase in CO_2 might be difficult to detect. However, the crabs retreated into their shells in response to tactile stimulation, which is obviously an ecologically important behavior to protect their body against physical attack by predators or competitors (Briffa and Elwood 2000; Briffa et al. 2008a; Hazlett and Bach 2010).

Ocean acidification can impair the foraging ability of animals by reducing their capacity to recognize prey odor or handle prey (Dodd et al. 2015; Kim et al. 2016). Olfactory cues are used for prey detection by many marine animals, and are perhaps even more important for deep-sea animals inhabiting a dark, relatively featureless environment. The toy octopus used in our study was undoubtedly a poor simulation of physical attacks by real octopi and could not provide the chemical cues that may elicit defensive behaviors cued by olfaction. If olfaction is an important mechanism for predator detection (Dicke and Grostal 2001) and is also affected by environmental hypercapnia, then behavioral adaptations used by hermit crabs may be affected by ocean acidification (Leduc et al. 2013). Acid-base regulation and $GABA_A$ receptor functioning related to olfaction may be impaired by ocean acidification (Clements and Hunt 2015).

Multiple cues (e.g. visual, chemical, and acoustic cues) can contribute to sensory abilities of marine animals. If one sense is impaired by ocean acidification, other senses may be emphasized to provide some compensation (Devine et al. 2012). Without chemical or visual cues that could be important to detect predators, tactile cues used for predator recognition may be essential. Based on the results of this study, decision-making by deep-sea hermit crabs related to withdrawal into its shell to avoid a predator or subsequent reemergence would not be seriously impaired by ocean acidification.

The short acclimation period used in this study may have affected the behavior of hermit crabs. To measure the boldness of the hermit crabs, we transferred each crab from a 1 L jar to 10 L aquarium and allowed the crab to acclimate, undisturbed, for 5 min. By observing the normal behavior of the crabs, we assumed that 5 min. would be sufficient for them to adjust to new surroundings. However, this procedure might act as a confounding factor to influence the crabs' response to the simulated predatory attack, and may explain why the time for reemergence was slightly longer prior to exposure to the pH treatment. As the crabs are repeatedly placed in a new environment and exposed to simulated attacks, they may adjust their behavior to reemerge more quickly.

Though reduced pH seawater did not change the boldness response of the crabs, a closely related study found that exposure to low pH waters led to a transient increase in metabolic rates and impaired olfactory behavior, including antennular flicking and prey detection (Kim et al. 2016). Furthermore, there was higher individual variation for the speed of antennular flicking, prey detection, and respiration rates at lower pH, thereby suggesting that populations may be able to adapt to future acidification. However, in our experiment, the shells of gastropods used for shelter by hermit crabs were corroded after 5 months in pH 7.6 seawater and highly corroded in pH 7.1. The mineralogy of gastropod shells (principally calcite and aragonite) are susceptible to dissolution in undersaturated waters typical of the deep Northeastern Pacific, and future ocean acidification will promote degradation of most shells in these habitats. Therefore, for the persistence of this population, the availability of gastropod shells for their habitats is another concern. The scope of consequences of future ocean acidification and related changes in ocean conditions for deep-sea animals is not well understood, but will involve the direct physiological and behavioral responses of individuals, as well as cascading indirect changes driven by population and community interactions within whole ecosystems.

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