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Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates

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Summary

Ocean acidification is now recognized as a threat to marine ecosystems; however, the effect of ocean acidification on fertilization in marine organisms is still largely unknown. In this study, we focused on sperm flagellar motility in broadcast spawning reef invertebrates (a coral and a sea cucumber). Below pH 7.7, the pH predicted to occur within the next 100 years, sperm flagellar motility was seriously impaired in these organisms. Considering that sperm flagellar motility is indispensable for transporting the paternal haploid genome for fertilization, fertilization taking place in seawater may decline in the not too distant future. Urgent surveys are necessary for a better understanding of the physiological consequences of ocean acidification on sperm flagellar motility in a wide range of marine invertebrates.

Keywords: Fertilization, Ocean acidification, Reef invertebrates, Sperm flagellar motility

Introduction

Ocean acidification, caused by increasing atmospheric carbon dioxide (CO_2) concentrations, is a future threat to marine ecosystems (Orr *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007). Previous studies suggested that ocean pH has already declined by 0.1 unit over the past century, and ocean pH could be decreased by 0.3–0.4 units by the end of the century (Caldeira & Wickett, 2003; Orr *et al.*, 2005). The potential impact of ocean acidification on calcification by marine organisms such as corals has been well recognized (Raven *et al.*, 2005; reviewed in Kleypas *et al.*, 2006). However, other physiological responses including fertilization to ocean acidification in these marine organisms remain to be elucidated (Raven *et al.*, 2005; Kleypas *et al.*, 2006).

Many sessile marine invertebrates release their gametes into the sea to undergo fertilization. If sperm lose their ability to find eggs in the vast extent of the sea, the life of marine organisms is potentially limited. Sperm flagellar motility, which is indispensable for fertilization, is regulated by an elevation of intracellular sperm pH (pHi) (Christen *et al.*, 1982; Lee *et al.*, 1983; Nakajima *et al.*, 2005). Increasing external CO_2 causes acidification of body fluids and changes in ion balances within marine organisms (Raven *et al.*, 2005); thus, future ocean acidification may influence sperm flagellar motility in marine organisms. However, no studies have investigated how fertilization in corals is likely to be affected by ocean acidification, and few have investigated other marine invertebrates (Kurihara & Shirayama, 2004; Havenhand *et al.*, 2008). Therefore, we examined the effects of CO_2-induced acidification on sperm motility in two common, broadcast-spawning invertebrates that inhabit the coral reefs of Okinawa, Japan.

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Table 1 Summary of physical and chemical conditions in each experimental aquarium (SWS) during the pH adjusted experiments.

[a]pH_{SWS}	[a] °C	HCO_3^- (μmol/kg)	CO_3^- (μmol/kg)	pCO_2 (ppm)	Ω_{Arag}
8.03 ± 0.03	26.8 ± 0.5	1760–1815	195–220	400–475	3.2–3.5
7.77 ± 0.05	26.8 ± 0.5	1960–2020	115–140	775–1005	1.9–2.3
7.69 ± 0.06	26.8 ± 0.5	2000–2065	95–125	930–1260	1.6–2.0
7.64 ± 0.12	26.8 ± 0.5	1995–2115	75–125	905–1660	1.2–2.0
7.31 ± 0.11	26.8 ± 0.5	2150–2205	40–60	2115–3585	0.6–1.0
6.55 ± 0.11	26.8 ± 0.5	2270–2285	7–10	12600–21100	0.1–0.2

The carbon parameters were calculated based on pH_{SWS}, temperature, salinity 34.0 and the assumed total alkalinity of 2300 μmol/kg/Ω_{Arag}, aragonite saturation state. [a]Means ± SD.

Materials and methods

Animals

Gravid coral colonies (*Acropora digitifera*) were collected from a fringing reef near Oku fishery port in Okinawa Island, Japan. The sea cucumber, *Holothuria* spp. was collected at Sesoko Island, in the northern part of Okinawa, Japan. The animals were maintained in a running seawater tank under natural light conditions at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. The coral spawned around the full moon in May, June, and July, 2008. The coral gametes were collected after spawning according to Morita *et al.* (2006). The sea cucumber sperm were collected by squeezing the gonads according to Morita *et al.* (2009).

Preparation of pH-adjusted seawater

To prepare pH-adjusted seawater, we used a pH-stat system similar to that described by Leclercq *et al.* (2002). Seawater was filtered by an inline filter system (0.45 μm). Filtered seawater was bubbled with pure CO_2 to specifically adjust the pH of the seawater using a controller connected to a pH electrode (Micro-pH, Aquabase). The pH conditions of the seawater were adjusted to pH 6.6, 7.3, 7.6, 7.7, 7.8, and 8.0 based on the total hydrogen ion concentration pH scale. The pH conditions of pH 7.3 and 7.6 were predicted for year 2100 and year 2200 by geochemical models (Caldiera & Wickett, 2003). In order to clarify whether the response of corals to low pH conditions is linear or non-linear, an extreme condition of pH 6.6 was included in our experiments. The pH controller was adjusted using a solenoid valve that opens when pH rises to 0.01 higher than the desired level, thereby injecting pure CO_2 from the compressed CO_2 tanks. The temperature was held at 26.3–27.3 °C, which is the typical water temperature during coral spawning season in Okinawa (Suwa *et al.*, 2008).

Aquaria (12 l) were filled with seawater adjusted to each pH value. Each aquarium was established as a flow-through system. The stability of the pH in each aquarium was confirmed daily using a pH meter connected to a combined glass/reference pH electrode (713 pH Meter, Metrohm), which were calibrated against the total hydrogen ion concentration pH scale buffers: TRIS and AMP (Dickson *et al.*, 2007). The chemical and physical conditions of each treatment are summarized in Table 1. The pH, temperature and mean salinity (34.0) were measured, and the aragonite saturation state was estimated from these parameter values and the mean total alkalinity of 2300 μmol/kg reported for the region (Fujimura *et al.*, 2001) by using the computer program CO2SYS (Lewis & Wallace, 1998).

Motility assessment

Sperm flagellar motility was assessed with a microscope equipped with a dark field condenser. We placed 2–3 eggs in 100 μl pH-adjusted seawater taken from aquaria prepared as described above on the slide glass and added 0.5 μl sperm suspension (about 10^7 sperm/ml) there immediately. Flagellar motility was initiated in response to the eggs. Sperm movements were recorded in digital video by a CCD camera (63W1N, MINTRON) attached to the microscope. Sperm movements were captured with Premier 6.5 (Adobe systems) or I-movie (Apple), and sperm trajectories were traced with NIH image software (http://rsb.info.nih.gov/nih-image/). Flagellar motility was assessed from the captured movie using I-movie from five individuals of each species with 2 s of filming each.

Results and discussion

Sperm motility of a coral (*A. digitifera*) and a sea cucumber (*Holothuria* spp.) was assessed at pH 6.6 to pH 8.0 on a total hydrogen ion concentration pH scale, as described by Morita *et al.* (2006). In *A. digitifera*, even relatively small decreases in ambient pH (0.2 to pH 0.4 pH) resulted in significant decreases in sperm flagellar motility (69% of sperm were motile at pH 8.0, 46% at pH 7.8, less than 20% at pH ≤ 7.7) (Fig. 1A, C). Such effects should be viewed in the context of conservative estimates that decreases of 0.2

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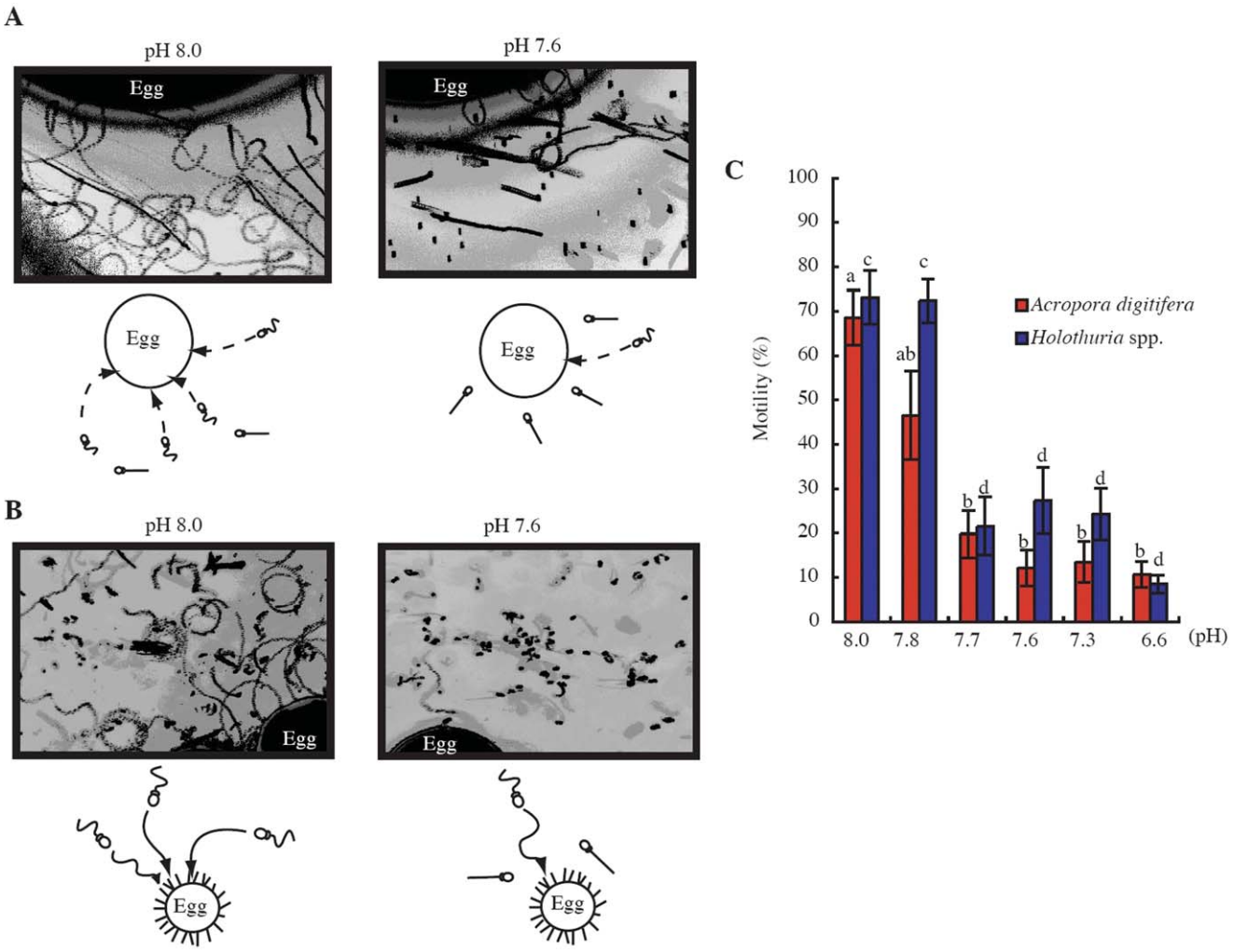

Figure 1 Trajectories of swimming sperm under different pH conditions. Sperm movements of (*A*) *Acropora digitifera* and (*B*) *Holothuria* spp. were recorded during 2-s periods. (*C*) Percentage of sperm motility in *A. digitifera* and *Holothuria* spp. in several pH conditions. Values are mean ± SE, n = five of each species with 2 s of filming each. Letters indicate statistical significance (a:b, $p < 0.0001$; ab:b, $p < 0.01$; c:d, $p < 0.001$ ANOVA/Bonferroni test).

to 0.4 units in ocean pH are likely within 100 years (Orr *et al.*, 2005). In *Holothuria* spp., a significant decrease in flagellar motility was also detected with reductions in pH (73% of sperm were motile at pH 8.0, 72% at pH 7.8, <30% at pH ≤ 7.7) (Fig. 1B, C). Many sessile marine organisms release their gametes into the water column for fertilization, resulting in a rapid dilution of sperm concentration (Levitan & Petersen, 1995). Therefore, a slight decrease in sperm motility could seriously threaten their life cycle, due to inefficiency with respect to fertilization. Whether corals can adapt to future ocean acidification is still under debate (Baird & Maynard, 2008), but relatively long generation times and a higher sensitivity of sperm to ocean acidification may restrict their potential for adaptation.

In general, sperm of broadcast spawners including the coral *A. digitifera* and the sea cucumber *Holothuria* spp. do not become motile just after they are released into the sea, but are motile in the vicinity of eggs (Miller, 1985; Morita *et al.*, 2006, 2009). Egg-derived compound(s) attach to the sperm and induce several signal cascades including motility activation and chemotaxis (Yanagimachi, 1957; Miller, 1985; Yoshida *et al.*, 1993; Coll *et al.*, 1994; Eisenbach & Tur-Kaspa, 1994; Morita *et al.*, 2006). During the course of motility activation, a difference between the extracellular and intracellular region is utilized as a signal to activate sperm flagellar motility in response to the egg-derived compound(s). A pHi causes activation of flagellar motility in many marine invertebrates, including sea urchin, starfish, sea cucumber,and coral (e.g. Christen *et al.*, 1982; Nakajima *et al.*, 2005; Morita *et al.*, 2009). If sperm from many marine invertebrates require a pHi to activate their flagellar motility, then it

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might be reasonable to assume that sperm could lose their ability to move as a result of a decline of ambient pH (pHo), such as occurs with acidification of seawater.

Nevertheless, sea urchin sperm motility is suppressed in acidic seawater (Havenhand *et al.*, 2008) because sea urchin sperm flagellar motility is activated after release into seawater. In the gonad, the pH is maintained below pH 7.3 by stable CO_2 tension (Johnson *et al.*, 1983), and sperm flagellar motility is activated when sperm are released into seawater accompanied by a reduction of intracellular proton concentration, causing a pHi up to 7.5 to 7.6. As a consequence, pHi induces the activation of dynein ATPase and mitochondrial respiration resulting in the initiation of motility (Christen *et al.*, 1982). In contrast, coral and sea cucumber sperm do not become motile after being released into seawater (Miller *et al.*, 1985; Morita *et al.*, 2006, 2009). In these species, egg-derived substance(s) stimulate protein phosphorylation of flagellar proteins leading to the activation of motility (e.g. Morita *et al.*, 2009). Therefore, there is a diversity of motility activation reactions among marine invertebrates that would be exposed to acidified seawater. Analysis of flagellar motility in several marine organisms in response to acidified seawater is required to describe a specific effect of ocean acidification on sperm motility. These surveys will provide us with an estimate of which marine organisms are likely to be endangered by ocean acidification.

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