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Sperm motility of the scleractinian coral *Acropora digitifera* under preindustrial, current, and predicted ocean acidification regimes

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ABSTRACT: Ocean acidification caused by the uptake of anthropogenic CO_2 in the oceans negatively affects the early life stages of corals by reducing their calcification rate. Acidification also inhibits the sperm motility of corals, potentially affecting fertilization success. We investigated the effects of different pCO_2 (partial pressure of CO_2) conditions on the sperm motility of *Acropora digitifera.* Using a pCO_2-control system, we maintained pCO_2 at concentrations from preindustrial and present-day levels up to the level predicted by the year 2100 (300, 400, and 1000 ppm, respectively). Our results indicated that ocean acidification has the potential to suppress the sperm flagellar motility of *A. digitifera*. Furthermore, sperm motility will likely decline by ~30%, which may impact fertility, if the sensitivity of sperm motility to decreasing pH cannot adapt over a span of ~90 yr.

KEY WORDS: Ocean acidification · Scleractinian corals · *Acropora* · Sperm motility

INTRODUCTION

Atmospheric CO_2 is increasing due to anthropogenic inputs of CO_2. Levels have increased by ca. 32% from the preindustrial era (from 280 µatm to ~380 µatm; Houghton et al. 2002), which has caused a change in seawater acidity of 0.1 pH units (IPCC 2007). Seawater pH is expected to decrease an additional 0.15 to 0.35 units by the end of this century (IPCC 2007).

Coral-reef builders may be negatively affected by a decrease in ocean pH (Hoegh-Guldberg et al. 2007). Acidification, resulting in an altered carbonate ion balance, may compromise the calcification of corals (e.g. Cohen et al. 2009, Jury et al. 2010, Suwa et al. 2010). In addition to the effects on calcification, ocean acidification may detrimentally affect coral fertilization (Albright et al. 2010) in the form of impaired sperm flagellar motility (Morita et al. 2010), settlement (Albright et al. 2010, Nakamura et al. 2011), and the establishment of symbiosis through slowed symbiont acquisition (Suwa et al. 2010). However, whether the imminent increased acidification will be associated with increased deleterious effects on early life stages is unclear.

Acropora digitifera is recognized as a species likely to be vulnerable to ocean acidification. In previous studies, *A. digitifera* has demonstrated some vulnerability to acidified seawater during early life stages, i.e. the fertilization process (Morita et al. 2010), settlement processes (Nakamura et al. 2011), and post-settlement processes (Suwa et al. 2010). Using this species, we examined the effects of different partial pressures of CO_2 (pCO_2) on fertilization success by measuring the sperm motility of corals. The pCO_2 levels were regulated by a pCO_2 stat system using 3 treatments of pCO_2 levels representing the preindustrial period, present day (control), and predicted pCO_2 for the year 2100 (the A1FI scenario; IPCC 2007).

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MATERIALS AND METHODS

Aquarium setup

A pCO_2-control system (Fujita et al. 2011, Hikami et al. 2011) was used for multiple pCO_2 exposure experiments. By adding CO_2 gas, the pCO_2 conditions of the seawater were adjusted to 300 (preindustrial levels), 400 (control), and 1000 ppm (the predicted level for the year 2100). Aquaria (12 l) were filled with running seawater adjusted to each pCO_2 value. A pCO_2 monitoring system (CO2-07, Kimoto Electron) monitored the pCO_2 level every hour using a non-dispersive infrared analyzer (LI-840, Li-Cor). The water temperatures in the experimental aquaria were recorded at 15 min intervals using data loggers (Water Temp Pro, Onset). Temperatures were maintained at 27°C, which is the typical water temperature during the coral spawning season in Okinawa (Loya et al. 2001) using 200 W heaters and thermostats (ET-30B, Kotobuki). The stability of the pH in each aquarium was confirmed using a pH meter connected to a combined glass/reference pH electrode (713 pH meter, Metrohm), which was calibrated against total hydrogen ion concentration (pH_t) pH-scale buffers: tris(hydroxymethyl)aminomethane (TRIS) and 2-amino-2-methyl-1-propanol (AMP) (Dickson et al. 2007). The chemical and physical conditions of each treatment are summarized in Table 1. The pH was measured once every 2 d. The aragonite saturation state was estimated from pH, salinity, temperature, and mean total alkalinity using the computer program CO2SYS (Lewis & Wallace 1998). The mean total alkalinity of 2235 µmol kg^{-1} was calculated from the values measured for 4 d prior to the experiment.

Sperm experiment

Gravid colonies of *Acropora digitifera* were collected prior to spawning from a fringing reef at Sesoko Island, Japan (26° 38′ N, 127° 51′ E). The colonies were kept in outdoor running seawater tanks under natural conditions (water temperature, light, and pCO_2) at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan, until spawning took place.

Gametes of *Acropora digitifera* were collected on spawning nights during the full moon in June 2009. Because gametes of *A. digitifera* form egg-sperm bundles at their release, gamete bundles were washed with filtered seawater to separate sperm from eggs.

Sperm flagellar motility was investigated following Morita et al. (2010). Because egg extracts initiate sperm flagellar motility (M. Kitamura & M. Morita unpubl. data), egg extracts were used to estimate the effect of acidified seawater on motility initiation. Egg extracts were prepared according to Morita et al. (2009) using eggs collected 1 yr before. Eggs were kept in methanol because the fraction that activates sperm motility is stable for at least 3 yr at –30°C. Substances from eggs were partitioned into 3 layers (a water layer, 90% methanol layer, and hexane layer) as follows: a volume of eggs (10 ml) was incubated overnight in 3 volumes of 99.5% ethanol (30 ml) at 4°C. The egg suspensions were centrifuged at 10 000 × *g* for 10 min at 4°C to collect the supernatant, and the supernatant was dried using a vacuum evaporator centrifuge (VEC-310; Iwaki). The dried supernatant was partitioned into a water layer and ethylacetate layer, and both layers were air-dried. Then, 100 µl of filtered seawater was added to the water layer for the following experiment.

Sperm motility activation was observed as follows: 50 µl of pCO_2-adjusted seawater was placed on a glass slide, and immediately, 1 µl of sperm suspension (2×10^7 to 8×10^7 cell ml^{-1}) was suspended into the seawater, followed by 1 µl of the water layer of the egg suspension to activate sperm motility. Sperm motility activation was recorded using a video recorder (DCR-TRV70, Sony) and a CCD camera (63W1N, Mintron) mounted on a microscope equipped with phase contrast or a dark-field condenser (Opthiphoto, Nikon). We began video recordings immediately after the addition of sperm activation substances. The recordings were continued for 3 min. The percentage of motile spermatozoa was estimated based on video recordings captured using iMovie 5.02 software (Apple). To calculate the percentage of motile sperm, the total number of spermatozoa in the optic field was counted. In other words, we counted the number of sperm moving just

Table 1. Physical and chemical conditions (mean ± SD; n = 6) in each experimental aquarium. The carbon parameters were calculated based on pH_t (total hydrogen ion concentration scale), temperature, a salinity of 34.0, and a total alkalinity of 2235 µmol kg^{-1}. Ω_{Arag}: aragonite saturation state

pH_t	Temp. (°C)	pCO_2 (µatm)	HCO_3 (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	Ω_{Arag}
8.17 ± 0.03	27.1 ± 0.1	225–280	1500–1580	265–300	4.2–4.8
8.05 ± 0.03	27.1 ± 0.1	330–395	1640–1705	215–240	3.4–3.8
7.74 ± 0.03	27.1 ± 0.3	790–945	1900–1945	115–135	1.9–2.2

above the glass slide within the objective field of the microscope; thus, measuring the exact sperm concentration in the experiment was difficult. We then counted the number of immotile spermatozoa to calculate the percentage of motile cells by subtracting the number of immotile sperm from the total number of sperm and dividing by the total number of sperm.

The differences in the percentage of motile sperm among pCO_2 levels were examined using 1-way ANOVA and Tukey's HSD tests for multiple comparisons. The data were arcsine transformed to meet the assumptions of normality. The normality of the data was checked using Shapiro-Wilks tests (300 ppm: $p = 0.66$; 400 ppm: $p = 0.86$; 1000 ppm: $p = 0.06$), and homogeneity of variance was assessed using Levene's test ($p = 0.094$).

RESULTS AND DISCUSSION

The percentage of motile sperm of *Acropora digitifer*a declined with increasing pCO_2 (ANOVA, $F_{2,12} = 4.38$, $p = 0.04$; Fig. 1), significantly decreasing from the preindustrial level (300 ppm, 96.6% motility) to the near-future level (1000 ppm, 60.1% motility) (Tukey's HSD test, $p < 0.05$). However, only a slight decrease was observed from the preindustrial level to the present level (400 ppm, 90.0% motility) and from the present level to the near-future level (neither comparison was statistically significant; Tukey's HSD test, $p > 0.05$). These results demonstrated a progressive decrease in the number of motile sperm with increasing CO_2.

An increase in intracellular pH is generally considered to activate sperm motility for many marine invertebrates (e.g. sea urchins, Christen et al. 1982; starfish, Nakajima et al. 2005; sea cucumbers, Morita et al. 2009). Accordingly, with a higher environmental pH at the preindustrial level, the sperm of *Acropora digitifera* were more active, even though the difference in motility was not statistically significant.

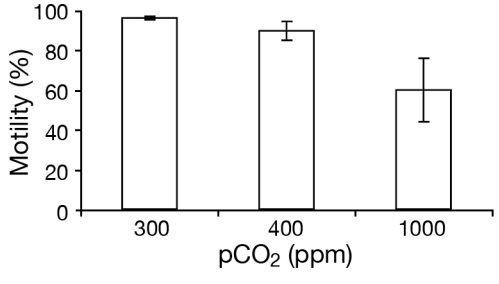

Fig. 1. *Acropora digitifera*. Sperm motility (%) under 3 pCO_2 conditions at 27°C. Values are mean ± SE, n = 5

This suggests that sperm may have acclimated to present-day seawater conditions, which are already acidified relative to the preindustrial era. In contrast, with a decrease in pH to the near-future level, the number of motile sperm significantly decreased. These results demonstrate that a decline of pH from the preindustrial to present level could limit the ability of sperm to move. Furthermore, the potential of coral sperm to acclimate to near-future acidified seawater conditions may be restricted (Morita et al. 2010).

In marine broadcast spawners, which release their gametes into the water column, diffusion of sperm makes it difficult to retain appropriate sperm concentrations for fertilization, a process called 'sperm limitation' (e.g. Levitan & Petersen 1995, Yund 2000). Sperm and egg interactions that lead to fertilization depend on random sperm–egg collision (Vogel et al. 1982, Styan 1998). Thus, when the number of sperm is limited, fertilization strongly depends on chemical cues to activate sperm motility (e.g. Bolton & Havenhand 1996, Eisenbach 1999, Jantzen et al. 2001). In the present study, a reduction of sperm motility activation via egg-derived substance(s) was observed with rises in pCO_2 concentration (Fig. 1), indicating that the amount of sperm available for fertilization decreased due to elevated CO_2.

The percentage of sperm motility reflects the number of available sperm for fertilization. Fertilization is not affected when higher numbers of sperm are present in the water column. Albright et al. (2010) demonstrated for the coral species *Acropora palmata* that sperm concentration declined from 3.21×10^6 to 3.20×10^5 sperm ml^{-1} as ocean acidification increased. Although we did not test the effects of pCO_2 concentration on fertilization rates for various sperm concentrations, we did use a higher sperm concentration (1.05 ml of 2×10^7 to 8×10^7 sperm ml^{-1}) than Albright et al. (2010) and still observed a significant decline in sperm motility. Therefore, a reduction in fertilization success at high pCO_2 concentrations may be related to sperm motility suppression with increased pCO_2 concentration; however, this issue remains controversial. Although the effects of sperm motility on fertilization have not yet been confirmed *in situ*, a reduction in sperm motility would affect the reproduction of hermaphroditic coral. For example, simultaneous hermaphroditic invertebrates can alter sex allocation, which means that eggs/sperm production exhibits high plasticity and is condition-dependent (e.g. Trouve et al. 1999, Schärer & Ladurner 2003). The fertilization rate is not expected to decrease if hermaphroditic coral create twice the

number of sperm via reductions in egg production (changes in sex allocation). The effect of ocean acidification on fertilization success remains uncertain, but basic information about sperm motility is very useful for predicting the effects of ocean acidification on coral reproduction.

In conclusion, our results demonstrated a progressive decrease in sperm motility with increasing ocean acidification, from preindustrial to near-future levels. Moreover, if the pH sensitivity of sperm motility of the coral *Acropora digitifera* cannot adapt over the next several decades, the percentage of motility will likely decline by ~30%, which may impact fertility.

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