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Baseline

Sperm swimming in the polychaete *Galeolaria caespitosa* shows substantial inter-individual variability in response to future ocean acidification ☆

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ABSTRACT

The rapidity of ocean acidification intensifies selection pressure for resilient phenotypes, particularly during sensitive early life stages. The scope for selection is greater in species with greater within-species variation in responses to changing environments, thus enhancing the potential for adaptation. We investigated among-male variation in sperm swimming responses (percent motility and swimming speeds) of the serpulid polychaete *Galeolaria caespitosa* to near- (ΔpH −0.3) and far-future ocean acidification (ΔpH −0.5). Responses of sperm swimming to acidification varied significantly among males and were overall negative. Robust sperm swimming behavior under near-future ocean acidification in some males may ameliorate climate change impacts, if traits associated with robustness are heritable, and thereby enhance the potential for adaptation to far-future conditions. Reduced sperm swimming in the majority of male *G. caespitosa* may decrease their fertilization success in a high CO_2 future ocean. Resultant changes in offspring production could affect recruitment success and population fitness downstream.

The rapidity of anthropogenic marine climate change intensifies the pressure for marine organisms to adapt and survive (Hoegh-Guldberg and Bruno, 2010; Doney et al., 2012; Zeebe, 2012). Selection for phenotypes resilient against environmental changes may increase a species' adaptation potential, if traits associated with robustness are heritable. In such cases, the scope for selection will be greater in species that exhibit naturally large inter-individual variation in responses (Sunday et al., 2011; Foo et al., 2012; Schlegel et al., 2012).

Climate change impacts on vulnerable gametes are particularly likely to have flow-on effects, especially in broadcast spawners (Hofmann et al., 2010; Kroeker et al., 2010). Here, selection against susceptible phenotypes may, if heritable, quickly reduce the genetic composition and diversity of subsequent life stages. A resultant gene bottleneck could have severe consequences for overall species fitness (Reed and Frankham, 2003; Frankham, 2005).

An increasing number of studies are focusing on responses of gametes to future ocean conditions across a range of broadcast spawning species (Wicks and Roberts, 2012; Gazeau et al., 2013), particularly in echinoderms (e.g., Caldwell et al., 2011; Reuter et al., 2011; Schlegel et al., 2012). With the exception of a recent study by Lewis et al., (2012), polychaetes have been largely overlooked. This is perplexing as they are common foundation species that modify environments and enhance biodiversity (Smith et al., 2005), and are important as fouling organisms (Bulleri et al., 2005), and soft sediment bioturbators (Coleman and Williams, 2002).

We investigated the sperm swimming behavior of the serpulid polychaete *Galeolaria caespitosa* (Lamarck 1818) under CO_2-induced ocean acidification. *G. caespitosa* is a tube building filter feeder that dominates the mid intertidal region on moderate to extremely exposed rocky shores along the temperate Australian intertidal environment (Edgar, 1997; Bulleri et al., 2005). Due to its tolerance to hyposaline conditions, this species also commonly occurs in estuarine environments (Tait et al., 1984). *G. caespitosa* has a complex life history, where dioecious adults are reproductively mature during most months of the year. Gametes fertilize externally and develop into free swimming planktotrophic larvae that mature into demersal larvae (Andrews and Anderson, 1962; Marsden and Anderson, 1981). After settlement, larvae metamorphose into juveniles that build and reside in a carbonate tube

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cemented to the substrate (Smith et al., 2013). The fertilization kinetics are well documented for *G. caespitosa* (Kupriyanova and Havenhand, 2002; Kupriyanova and Havenhand, 2005; Kupriyanova, 2006). *G. caespitosa* is used as a model species in studies on the evolution of polyandry and sperm competition (e.g., Evans and Marshall, 2005; Styan et al., 2008; McLeod and Marshall, 2009) as well as in ecotoxicological assessments (Moran and Grant, 1993; Ross and Bidwell, 2001).

Here, we focused on among-male variation in sperm swimming responses to future ocean acidification. Following the A1FI scenario (IPCC, 2007), we exposed sperm to seawater conditions predicted for near- (pCO_2 = 970 μatm, year 2100) and far-future CO_2 scenarios future (pCO_2 = 1600 μatm, year 2300), and recorded impacts on the proportion of motile sperm and their swimming speeds. Based on a previous study on individual variation in sperm swimming in sea urchins (Schlegel et al., 2012), we hypothesized that there will be substantial variation in the responses of swimming capabilities in individual sperm.

Filtered seawater (FSW; 0.22 μm filtered) was aerated with a CO_2/air mixture to achieve CO_2 treatments. Seawater temperature and salinity (Table 1) were measured for each replicate (n = 23) using an IQ Sensor net (MIQ/T2020, WTW). Microprocessor CO_2 injection units were set to maintain stable pH_{NBS} levels of 8.1 (controls, no CO_2 added; pCO_2 = 427 μatm), 7.8 (pCO_2 = 971 μatm) and 7.6 (pCO_2 = 1597 μatm), following the A1FI scenario (IPCC, 2007). Total alkalinity was determined for every third replicate (n = 7) by titration (HI 3811 Alkalinity kit, Hanna Instruments), all other parameters of the CO_2 system were calculated using CO2-SYS (Lewis and Wallace, 1998) and the dissociation constants of Dickson and Millero, (1987) (Table 1).

Clumps of large *G. caespitosa* (tube openings of 2+mm diameter) were collected from intertidal rock platforms in Fairlight, Sydney, Australia (33°48′1″S, 151°16′3″E) in November and December 2011, and held in a recirculating seawater system at Macquarie University. Individuals were used in experiments within 48 h of collection.

Collection of gametes followed the protocol by Kupriyanova and Havenhand, (2002). Individual *G. caespitosa* were carefully removed from their calcareous tubes and inspected for ripeness. Individual males, characterized by creamy white lower abdomens, were placed into separate petri dishes. Removal of the males from their tubes caused instantaneous spawning in mature individuals. Males that did not immediately release gametes were discarded. Sperm from spawning individuals were collected with Pasteur pipettes from each male, and held "dry" on ice in Eppendorf tubes (one for each individual) until immediately prior to use (within 15 min of release). A total of 23 mature males were tested.

Sperm motility experiments were conducted in a temperature-controlled room at 20 ± 0.5 °C and followed established protocols (Havenhand et al., 2008; Havenhand and Schlegel, 2009; Schlegel et al., 2012). "Dry" sperm (~0.5–1 μl) were diluted in 1.5 ml of seawater of each pH immediately before use (final concentrations of $1–2 \times 10^4$ sperm μl^{-1}). Ten replicate sperm suspensions were freshly prepared for each pH treatment and for each male. A drop of sperm suspension (~60 μl) was placed between an albumin-coated microscope slide and cover slip, separated by a 0.75 mm thick O-ring. Sperm movements were video recorded immediately after suspension using a digital video camera (SMX-160; at 25 frames s^{-1}) mounted on a compound microscope (Olympus BX51). Videos were post-processed and 2s-clips were analyzed using CellTrak 1.3 (Motion Analysis Corporation) for the proportion of motile sperm (defined as sperm moving faster than 15 μm s^{-1}) and their swimming speed. A total of 10 replicate recordings were made for 10 separate sperm suspensions for each male and pH treatment.

All percentage data were arc-sin transformed prior to statistical analyses (Quinn and Keough, 2002). Data were assessed for homogeneity of variances among individuals using Levene's test, before using two-way ANOVA (pH fixed, male random) to test pH effects on percent motility and speed of motile sperm. Differences between means were compared *post hoc* using Tukey's test. Among-male responses were assessed using logarithmic response ratios (LnRR; natural log of treatment response divided by control response; Hedges et al., 1999). Upper and lower boundaries for 95% confidence intervals around mean LnRRs were determined by bootstrapping in R (100,000 iterations). All other analyses were carried out using SPSS™.

CO_2-induced ocean acidification significantly reduced the overall proportion of motile sperm and their swimming speeds compared to present day (ambient) conditions (Fig. 1A, Table 2). Responses among individual males, however, varied substantially (Fig. 1B). While sperm from the majority of *G. caespitosa* males were less motile and slower under near-future conditions compared to present ambient conditions (ΔpH −0.3; Fig. 1B, Table 3), sperm from some males (n = 7) showed either slightly increased motility and/or swimming speed, or no change in these parameters. Only few males (n = 3) showed robust sperm swimming under far-future conditions (ΔpH −0.5; Table 3).

For percent sperm motility, upper and lower bound 95% confidence intervals around individual log response ratios (LnRR) were equivalent to changes of +4.6% to −38.7% at ΔpH −0.3 (Fig. 1B); and of −13.4% to −46.6% at ΔpH −0.5.

For speed of motile sperm, 95% confidence intervals around LnRRs were equivalent to changes of +0.7% to −24.8% at ΔpH −0.3; and of −9.2% to −38.2% at ΔpH −0.5.

We found substantial, and significant, variation in sperm swimming responses among single males of *G. caespitosa* to CO_2-induced ocean acidification. Overall percent sperm motility and sperm swimming speeds declined significantly under ocean acidification. Sperm from a minority of males seemed robust to near-future acidification scenarios (ΔpH −0.3), showing no, or even positive, responses. Even fewer males were robust to far-future acidification scenarios (ΔpH −0.5). If this robustness to near-future conditions is heritable, it could act as a base for adaptation to far-future conditions (Sunday et al., 2011), provided that adaptation can occur within the relatively short time frame of predicted future ocean acidification.

The inter-male variability we observed was not unexpected: *G. caespitosa* naturally exhibit high intra-specific variation in sperm swimming behavior (Kupriyanova and Havenhand, 2002, Fig. 1A). The extent to which this variability depends on seasonal changes in reproductive condition and temperature is unknown. Further, the substantial range in sperm responses among individuals to ocean acidification observed here – from highly positive to

Table 1
Seawater parameters. pH_{NBS}, temperature (T), salinity (Sal), and total alkalinity (A_T) were measured directly and used to calculate partial CO_2 pressures (pCO_2) and seawater saturation states for calcite (Ω_{Ca}) and aragonite (Ω_{Ar}) using CO2-SYS (see text). Data are means ± S.E. n = 23 for pH, T and Sal. n = 7 for A_T.

pH_{NBS}	T (°C)	Sal	A_T (μeq kg^{-1})	pCO_2 (μatm)	Ω_{Ca}	Ω_{Ar}
8.10 ± 0.01	20 ± 0.5	35 ± 0.1	2029 ± 5	427	3.40	2.21
7.80 ± 0.01	20 ± 0.5	35 ± 0.1	2029 ± 5	971	1.90	1.24
7.60 ± 0.01	20 ± 0.5	35 ± 0.1	2029 ± 5	1597	1.25	0.81