Baseline

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

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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 CO2 future ocean. Resultant changes in offspring production could affect recruitment success and population fitness downstream.

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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 CO2-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 reproduc-tively mature during most months of the year. Gametes fertilize externally and develop into free swimming planktrophic 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.
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 ecotoxological 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 AIFl scenario (IPCC, 2007), we exposed sperm to seawater conditions predicted for near- (*pCO2* = 970 μatm, year 2100) and far-future *CO2* scenarios (*pCO2* = 1600 μatm, year 2300), and recorded impacts on the proportion of motile sperm and their swimming speeds. Based 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 *CO2*/air mixture to achieve CO2 treatments. Seawater temperature and salinity (Table 1) were measured for each replicate (*n* = 23) using an IQ Sensor net (MIQ/T2020, WTW). Microprocessor *CO2* injection units were set to maintain stable pH (9.9 ± 0.01) for every third replicate (*n* = 3) showed robust sperm swimming under future-future conditions (*ΔpH* = −0.5; Table 3). Clumps of large *G. caespitosa* (tube openings of 2+μm 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 × 10^6 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 25-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 males 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™.

**Table 1** Seawater parameters. pH (pH), temperature (T), salinity (Sal), and total alkalinity (A<sub>T</sub>) were measured directly and used to calculate partial CO2 pressures (*pCO2*) and seawater saturation states for calcite (Ω<sub>CaCO3</sub>) and aragonite (Ω<sub>CaCO3</sub>) using CO2-SYS (see text). Data are means ± S.E. *n* = 23 for pH, T and Sal, *n* = 7 for A<sub>T</sub>.

| pH (pH) | T (°C) | Sal | A<sub>T</sub> (μeq kg ^−1) | pCO2 (μatm) | Ω<sub>CaCO3</sub> | Ω<sub>CaCO3</sub> |
|---------|--------|-----|--------------------------|-------------|-------------------|-------------------|
| 8.10 ± 0.01 | 20 ± 0.5 | 35 ± 0.1 | 2029 ± 5 | 427 | 3.40 | 2.11 |
| 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 |
negative (Fig. 1B) – suggests that these responses are not reaction norms. Such large variation in responses increases the scope for selection of rare sperm phenotypes robust to future acidification (Pistevos et al., 2011; Sunday et al., 2011; Foo et al., 2012; Schlegel et al., 2012), which may contribute disproportionately more to subsequent generations. This selection may thus ameliorate ocean acidification effects on a species, if traits associated with acidification resistance are heritable. In this context, it is important to stress the need for adequately replicated studies on climate change impacts in order to accurately estimate the extent of inter-individual variation (Havenhand et al., 2010). Resilience to near-future climate change observed in the sperm of some males could act as a stepping stone for adaptation to far-future conditions, if gathering of advantageous alleles through recombination in subsequent generations can outrun the rapidity of predicted ocean acidification. Consequently, simultaneous selection against susceptible phenotypes could quickly reduce genetic diversity, with flow-on consequences for species fitness and competitive ability (Reed and Frankham, 2003; Frankham, 2005).

Changes in sperm swimming behavior affect fertilization success (Vogel et al., 1982; Styan and Butler, 2000; Styan et al., 2008). Positive relationships between fertilization success and sperm concentration – influenced by percent motility – as well as sperm swimming speeds have been reported for this species (Kupriyanova and Havenhand, 2002; Kupriyanova, 2006). Sperm swimming speeds are reported to be enhanced under increased water temperatures (Kupriyanova and Havenhand, 2005), and therefore future ocean warming could ameliorate acidification-related reductions in sperm swimming speeds, particularly during warmer summer temperatures (Hobday and Lough, 2011). For the majority of G. caespitosa, however, potential positive effects of ocean warming on sperm swimming speeds would likely be swamped by the substantial negative effects of ocean acidification on percent motility that we observed (Fig. 1). Naturally occurring sperm limitation scenarios (Williams et al., 1997) could thereby be exacerbated, decreasing reproductive output in affected males. Conversely, reduced sperm swimming under acidified conditions could increase sperm longevity due to lowered consumption of limited endogenous energy provisioning (Mita and Nakamura, 1998). Greater sperm longevity may increase chances of successful fertilization if sperm–egg-encounter rates remain sufficient over prolonged periods of time (Levitan, 2000; Marshall, 2002). Impacts of CO2-driven ocean acidification on sperm swimming behavior of G. caespitosa may interact with other acidification impacts on fertilization variables such as male–female compatibility, egg competition or polyspermy block efficiency (Evans and Marshall, 2005; Marshall and Bolton, 2007). Negative impacts of CO2–induced ocean acidification have also been reported for later life-history stages of serpulid tubeworms, such as weaker calcareous tubes.
(Chan et al., 2012; Smith et al., 2013). Resultant cumulative effects on reproductive success and survivorship are likely to exacerbate the rate or intensity of selection pressure of climate change.

Patterns of sperm swimming responses of *G. caespitosa* to CO₂-induced acidification observed here were similar to those of *Arenicola marina* sperm in lowered seawater pH (Pacey et al., 1994). Sperm activation in *A. marina* was delayed and sperm speed was reduced in HCl-acidified seawater (pH < 7.6). Interestingly, our findings are very different to those from a study on the related serpulid species *Pomatoceros lamarckii* (Lewis et al., 2012). Sperm speeds of *P. lamarckii* were robust to CO₂-induced pH reductions, percent motility was significantly reduced, but responses were non-linear. These findings may be explained by differences in experiment design and sample size (5 pooled assays (Lewis et al., 2012) vs 23 single individuals in this study). As outlined earlier, conducting adequately replicated studies will help to clarify whether these differences are caused by high inter-individual variability or differences in average responses between species.

In conclusion, the substantial inter-individual variation in sperm responses observed here may ameliorate effects of future climate change, if the traits that drive phenotype robustness are heritable. Sperm from some *G. caespitosa* will be better equipped to cope with acidification than others, creating ‘winners’ and ‘losers’ in a future acidified ocean (Schlegel et al., 2012). This observed resilience to near-future conditions could increase the ability of organisms to cope with acidification than others, creating ‘winners’ and ‘losers’. Consequently, further research on reproductive success and survivorship are likely to exacerbate potential consequences for biodiversity. Trends in Ecology & Evolution 17 (1), 259–280.

**References**

Andrews, J.C., Anderson, D.T., 1962. The development of the polychaete *Galeolaria caespitosa* Lamark (fam. Serpulidae). Proceedings of the Linnean Society of New South Wales 87 (2), 185–188.

Bulleri, F., Chapman, M.G., Underwood, A.J., 2005. Intertidal assemblages on seawns and vertical rocky shores in Sydney Harbour. Australia Austral Ecology 30 (6), 655–667.

Caldwell, G.S., Fitzler, S., Gillespie, C.S., Pickavance, G., Turnbull, E., Bentley, M.G., 2005. Ocean acidification takes sperm back in time. Invertebrate Reproduction and Development 41 (1–3), 21–26.

Chan, V.B.S., Li, C., Lane, A.C., Wang, Y., Lu, X.Y., Shih, K., Zhang, T., Thiyagarajan, V., Caldwell, G.S., Fitzer, S., Gillespie, C.S., Pickavance, G., Turnbull, E., Bentley, M.G., 2012. In situ measures of spawning synchrony and fertilization success in the serpulid polychaete *Galeolaria caespitosa*. Invertebrate Reproduction and Development 41 (1–3), 21–26.

Doney, S.C., Ruckelshaus, M., Duffy, J.E., Barry, J.P., Chan, F., English, C.A., Calipino, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlin, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D., 2012. Climate change impacts on marine ecosystems. Annual Review of Marine Science 4 (1), 41–42.

Edgar, G.J., 1997. Australian marine life. Reed Books, Kew.

Evans, J.P., Marshall, D.J., 2005. Male-by-female interactions influence fertilization success and mediate the benefits of polyandry in the sea urchin *Heliocidaris erythrogramma*. Evolutionary Ecology 19 (1), 106–112.

Foo, S.A., Dworjanyn, S.A., Poore, A.G.B., Byrne, M., 2012. Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: performance of early embryos. PLoS ONE 7 (8), e42718.

Frankham, R., 2005. Conservation biology: ecosystem recovery enhanced by genotypic diversity. Heredity 95 (3), 183.

Gazeau, F., Parker, L.M., Conne, S., Gattuso, J.-P., O'Connor, W.A., Martin, S., Pörtner, H.-O., Ross, P.M., 2013. Impacts of ocean acidification on marine shellfish. Marine Biology.

Havenhand, J.N., Butler, F.R., Thornydale, M.C., Williamson, J.E., 2008. Near-future levels of ocean acidification reduce fertilization success in a sea urchin. Current Biology 18 (15), 1651–1655.

Havenhand, J.N., Dupont, S., Quinn, G.P., 2010. Designing ocean acidification experiments to maximize inference. In: Riebesell, U., Faby, V.J., Hansson, L., Gattuso, J.-P. (Eds.), Guide to Best Practices for Ocean Acidification Research and Data Reporting. Luxembourg Publications Office of the European Union, pp. 67–80.

Havenhand, J.N., Schlegel, P., 2009. Near-future levels of ocean acidification do not affect sperm motility and fertilization kinetics in the oyster *Crassostrea gigas*. Biogeochemistry 86 (1–3), 309–317.

Hedges, L.V., Gurevitch, J., Curtis, P., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156.

Hobbay, A.J., Lough, J.M., 2011. Projected climate change in Australian marine and estuarine environments. Marine and Freshwater Research 62 (9), 1099–1114.

Hoegh-Guldberg, O., Bruno, J.F., 2010. The impact of climate change on the world’s marine ecosystems. Science 328 (5985), 1523–1528.

Holmang, G.E., Barry, J.P., Edmunds, P.J., Gates, R.D., Hutchins, D.A., Klinger, T., Sewell, M.A., 2010. The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective. Annual Review of Ecology Evolution and Systematics 41, 127–147.

IPCC, 2007. The 4th Assessment Report of the IPCC. UK, Cambridge.

Korotki, K.J., Kordai, R.L., Crum, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters 13, 1419–1434.

Kupriyanova, E., 2006. Fertilization success in *Galeolaria caespitosa* (Polychaeta: Serpulidae): genotypic variability and potential consequences for biodiversity. Trends in Ecology & Evolution 17 (1), 259–280.

Kupriyanova, E., Havenhand, J.N., 2002. Variation in sperm swimming behaviour and its effect on fertilization success in the serpulid polychaete *Galeolaria caespitosa*. Invertebrate Reproduction and Development 41 (1–3), 21–26.

Kupriyanova, E., Havenhand, J.N., 2005. Effects of temperature on sperm swimming behaviour, respiration and fertilization success in the serpulid polychaete, *Galeolaria caespitosa* (Annelida: Serpulidae). Invertebrate Reproduction and Development 48 (1–3), 7–17.

Levitan, D.R., 2000. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. Proceedings of the Royal Society of London Series B–Biological Sciences 267 (1443), 531–534.

Lewis, E., Clemow, K., Holt, W.V., 2012. Metal contamination increases the sensitivity of larvae but not gametes to ocean acidification in the polychaete *Pomatoceros lamarckii* (Quatrefages), Marine Biology.

Lewis, E. & Wallace, D. (1998). Program developed for CO₂ system calculations. Oak Ridge, Tennessee: ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy.

Marsden, J.R., Anderson, D.T., 1981. Larval development and metamorphosis of the serpulid polychaete *Galeolaria caespitosa* Lamark. Australian Journal of Marine and Freshwater Research 32, 667–680.

Marshall, D.J., 2002. In situ measures of spawning synchrony and fertilization success in an intertidal, free-spawning invertebrate. Marine Ecology Progress Series 236, 113–118.

Marshall, D.J., Bolton, T.F., 2007. Sperm release strategies in marine broadcast spawners: the costs of releasing sperm quickly. Journal of Experimental Marine Biology 210 (21), 3720–3727.

McLeod, L., Marshall, D.J., 2009. Do genetic diversity effects drive the benefits associated with multiple mating? A test in a marine invertebrate. PLoS ONE 4 (8), e6347.

Mita, M., Nakamura, M., 1998. Energy metabolism of sea urchin spermatozoa: an approach based on echinoid phylogeny. Zoological Science 15 (1), 1–10.

Mita, M., Nakamura, M., 1998. Energy metabolism of sea urchin spermatozoa: an approach based on echinoid phylogeny. Zoological Science 15 (1), 1–10.

Moran, P.J., Grant, T.R., 1993. Larval settlement of marine fouling organisms in polluted water from Port Kembla Harbour. Australia. Marine Pollution Bulletin 26 (9), 512–514.

Pacey, A., Cosson, J., Bentley, M., 1994. The acquisition of forward motility in the polychaete *Hydroides elegans* (Polychaeta: Serpulidae). Invertebrate Reproduction and Development 236, 113–119.

Quatrefages). Marine Biology.
Reed, D.H., Frankham, R., 2003. Correlation between fitness and genetic diversity. Conservation Biology 17 (1), 230–237.

Reuter, K.E., Lotterhos, K.E., Crim, R.N., Thompson, C.A., Harley, C.D.G., 2011. Elevated pCO₂ increases sperm limitation and risk of polyspermy in the red sea urchin Strongylocentrotus franciscanus. Global Change Biology 17, 163–171.

Ross, K.E., Bidwell, J.K., 2001. A 48-h Larval development toxicity test using the marine polychaete Galeolaria caespitosa lamarck (Fam. Serpulidae). Archives of Environmental Contamination and Toxicology 40, 489–496.

Schlegel, P., Havenhand, J.N., Gillings, M.R., Williamson, J.E., 2012. Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins. PLoS ONE 7 (12), e53118.

Smith, A.M., McGourty, C.R., Kregting, L., Elliot, A., 2005. Subtidal Galeolaria hystrix (Polychaeta: Serpulidae) reefs in Paterson Inlet, Stewart Island, New Zealand. New Zealand Journal of Marine and Freshwater Research 39 (6), 1297–1304.

Styan, C.A., Butler, A.J., 2000. Fitting fertilisation kinetics models for free-spawning marine invertebrates. Marine Biology 137 (5), 943–951.

Styan, C.A., Kupriyanova, E., Havenhand, J.N., 2008. Barriers to cross-fertilization between populations of a widely dispersed polychaete species are unlikely to have arisen through gametic compatibility arms-races. Evolution 62 (12), 3041–3055.

Sunday, J.M., Crim, R.N., Harley, C.D.G., Hart, M.W., 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. PloS ONE 6 (8), e22881.

Tait, N.N., Atapattu, D., Browne, R., 1981. Field and laboratory studies on salinity tolerance and osmotic behaviour in the polychaete Galeolaria caespitosa (Serpulidae). Australian Journal of Marine and Freshwater Research 32 (5), 769–774.

Wicks, L.C., Roberts, J.M., 2012. Benthic invertebrates in a high CO₂ world. Oceanography and Marine Biology: An Annual Review 50, 127–188.

Zeebe, R.E., 2012. History of seawater carbonate chemistry, atmospheric CO₂, and ocean acidification. Annual Review of Earth and Planetary Sciences 40, 141–165.
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