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Effects of ocean warming and acidification on fertilization in the Antarctic echinoid *Sterechinus neumayeri* across a range of sperm concentrations

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ABSTRACT

The gametes of marine invertebrates are being spawned into an ocean that is simultaneously warming and decreasing in pH. Predicting the potential for interactive effects of these stressors on fertilization is difficult, especially for stenothermal polar invertebrates adapted to fertilization in cold, viscous water and, when decreased sperm availability may be an additional stressor. The impact of increased temperature (2–4 °C above ambient) and decreased pH (0.2–0.4 pH units below ambient) on fertilization in the Antarctic echinoid *Sterechinus neumayeri* across a range of sperm concentrations was investigated in cross-factorial experiments in context with near future ocean change projections. The high temperature treatment (+4 °C) was also used to assess thermal tolerance. Gametes from multiple males and females in replicate experiments were used to reflect the multiple spawner scenario in nature. For fertilization at low sperm density we tested three hypotheses, 1) increased temperature enhances fertilization success, 2) low pH reduces fertilization and, 3) due to the cold stenothermal physiology of *S. neumayeri*, temperature would be the more significant stressor. Temperature and sperm levels had a significant effect on fertilization, but decreased pH did not affect fertilization. Warming enhanced fertilization at the lowest sperm concentration tested likely through stimulation of sperm motility and reduced water viscosity. Our results indicate that fertilization in *S. neumayeri*, even at low sperm levels potentially found in nature, is resilient to near-future ocean warming and acidification.

1. Introduction

Many marine invertebrates are broadcast spawners with fertilization in the water column where eggs and sperm are directly exposed to environmental conditions. A long history of ecotoxicology research shows that fertilization success is highly sensitive to changes in seawater quality and chemistry (Dinnel and Stober, 1987; Byrne et al., 2008; Byrne, 2010). Due to global change, marine gametes and fertilization are experiencing increased seawater temperature and acidification/pCO_2, the magnitude of which varies among regions (McNeil and Matear, 2008; Hobday and Lough, 2011; Mulvaney et al., 2012). Thus, experiments especially the stressor levels used, need to be placed in a regional, seasonal and habitat relevant context.

Increased temperature (up to a threshold) increases metabolism and is known to enhance fertilization success in marine invertebrates due to stimulation of sperm swimming, reduced seawater viscosity and by increasing sperm–egg contact (Hagström and Hagström, 1959; Mita et al., 1984; Clotteau and Dubé, 1993; Kupriyanova and Havenhand, 2005). In contrast, high pCO_2 may suppress gamete metabolism, although this is only reported to occur at very low pH (pH $<$ 7.0) (Johnson et al., 1982) and the response of sperm to this stressor is variable. Increased pCO_2 within the range of near future ocean acidification projections reduces sperm motility and/or swimming speed in an echinoid and a coral (Morita et al., 2009; Schlegel et al., 2012), has no effect on sperm swimming speed in an oyster (Havenhand and Schlegel, 2009), and actually increases swimming speed in a different echinoid species (Caldwell et al., 2011). Thus, although warming and acidification might be predicted to have an antagonistic interaction on sperm behavior, these studies show that the responses of this gamete are hard to predict. It is also not clear how the response of isolated

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sperm relates to how fertilization may be affected by increased temperature and acidification. In nature, free amino acids released by eggs and small peptides in egg jelly increase sperm respiration, internal pH, metabolism of phospholipids and swimming speed (Ward and Kopf, 1993; Miller, 1997; Riffell et al., 2002).

Inferences on how fertilization, a fundamental life history process, may fare in a changing ocean requires investigation of the interactive effect of warming and acidification in cross-factorial studies where both gametes are simultaneously exposed to these stressors. Most global change studies on marine invertebrate fertilization have focused on acidification as a single stressor while many early studies of gamete biology examined the impact of increased temperature (Reviews Byrne, 2010, 2012). Thus far, the interactive effects of warming and acidification on fertilization in a global change context have been investigated in cross-factorial studies with three mollusc, six echinoid and two coral species (Byrne et al., 2010a,b; Parker et al., 2010; Ericson et al., 2012; Albright and Mason, 2013; Chua et al., 2013). In optimal sperm conditions (~>80% fertilization in controls) experiments with oysters indicate vulnerabilities of fertilization to both pH and temperature (Parker et al., 2010), but this was not the case for sea urchins (Byrne et al., 2010a; Ericson et al., 2012). As marine invertebrate fertilization in nature may be challenged by sperm limitation (Levitan and Petersen, 1995; but see Yund, 2000), it is also important to assess the potential for interactive effects (negative or positive) of increasing temperature and acidification on fertilization at low sperm density. Thus far, two studies have investigated the combined effects of warming and acidification on fertilization at low sperm density (Byrne et al., 2010b; Albright and Mason, 2013). At low sperm concentration both stressors exhibited a negative effect on a coral (Albright and Mason, 2013), but not an echinoid (Byrne et al., 2010a).

In single stressor studies of the effects of ocean acidification on fertilization in optimal and low sperm concentrations, results are variable, reflecting species differences, population differences and different experimental approaches (Byrne, 2012). For the intertidal sea urchin *Paracentrotus lividus*, the responses of fertilization to pH differed depending on tide pool of origin of the adults, with gametes from parents that experience lower pH in the field being more resilient (Moulin et al., 2011). Fertilizations using gametes pooled from multiple parents, reflecting the multiple spawner scenario in nature, potentially the mean population response, can show resilience to near-future (ca. pH 7.8) ocean acidification conditions, even at low sperm concentrations (Byrne et al., 2010b). In contrast, tests that involve single dam-sire crosses indicate greater sensitivity to near-future ocean acidification, but are also much more variable (Havenhand and Schlegel, 2009; Schlegel et al., 2012; Foo et al., 2012; Albright and Mason, 2013). This variation is likely due to variation in egg–sperm compatibility and assortative mating (Levitan, 2012).

Multistressor studies on the impacts of ocean warming and acidification on fertilization in marine invertebrates have largely involved temperate coastal species where the resilience of fertilization may be associated with adaptation to fluctuating temperature and pH conditions experienced in nature (Byrne, 2012). Fertilization in species from less variable environments such as polar regions might be comparatively more sensitive. Polar marine organisms are among the most stenothermal in the world due to the stability of their environment over evolutionary time (Clarke, 1983). As a consequence, they are expected to be sensitive to the slightest of environmental perturbations, particularly early life history stages (Barnes and Peck, 2008). Several studies indicate negative impacts of ocean acidification and/or warming on embryos and larvae of polar species (Stanwell-Smith and Peck, 1998; Comeau et al., 2009; Ericson et al., 2010, 2012; Kawaguchi et al., 2010; Byrne et al., 2013; Yu et al., 2013).

Antarctica is among regions most at risk with respect to the impacts of ocean acidification and warming on biota (Barnes et al., 2006; Clarke et al., 2007; McClintock et al., 2008; McNeil and Matear, 2008; Sewell and Hofmann, 2011; Mulvaney et al., 2012) and so synergistic effects of these stressors may be particularly important. Here we investigated the impact of increased temperature (2 °C above ambient) and decreased pH (0.2–0.4 pH units below ambient) on fertilization in the Antarctic echinoid *Sterechinus neumayeri* across a range of sperm concentrations in cross-factorial experiments, in context with near future ocean change projections (A1F1, IPCC, 2007). Our high temperature treatment (4 °C above ambient = 5 °C), approaches lethal levels for development in *S. neumayeri* (Stanwell-Smith and Peck, 1998; Peck et al., 2004), was used to assess tolerance of fertilization to this level of warming. As part of the range of *S. neumayeri* are among the fastest warming regions on Earth, it is important to go beyond the acidification, single stressor approach to understand the potential impacts of ocean change on fertilization in this species.

S. neumayeri has a key trophic role in Antarctic marine ecosystems and has been used as a model species for Antarctic research due to its importance as a predator and grazer, and its abundant circumpolar distribution (Brey et al., 1995). In single stressor experiments, fertilization in *S. neumayeri* is resilient to decreased pH (pH 7.5–7.7) and a recent study indicated a negative effect of warming at pH 7.5 (Ericson et al., 2010, 2012). It is not known whether the interactive effects of these stressors will have a negative impact on fertilization in *S. neumayeri* at low sperm concentrations that may occur in nature (Levitan and Petersen, 1995). We followed methods used in recent studies of the potential population response of echinoids from warmer latitudes to facilitate comparisons (e.g. gametes from multiple males and females, three pH and three temperature levels in all combinations, activation of sperm in treatment conditions) (Byrne et al., 2010a).

The dynamics of sea urchin fertilization in Antarctica are slower than that for urchins from warmer latitudes (Stokes et al., 1996), likely influenced by low temperature and the high viscosity of cold seawater (Vogel, 1994). Thus, we expected that increased temperature would exert a strong influence on fertilization in sperm limiting conditions. For fertilization at low sperm concentrations we tested three hypotheses, 1) increased temperature enhances fertilization, 2) low pH reduces fertilization and, 3) due to the cold stenothermal physiology of *S. neumayeri*, temperature would be the more significant stressor.

2. Materials and methods

2.1. Specimen collection

S. neumayeri adults were collected from 1 m depth at Ellis Narrows, Prydz Bay near Davis Station, East Antarctica (68.62°S, 77.99°E). Specimens were collected during Dec–Jan 2011/12 to coincide with their peak summer spawning period. They were transported to Davis Station and were held in aquaria at ambient pH (8.0) and temperature (1 ± 0.5 °C) and fed macroalga (*Palmaria dicipens*) until used for experiments. During the austral summer prior to and after the breakup of sea ice, *S. neumayeri* near Prydz Bay experience sea surface temperatures ranging from −1.6 to 1.1 °C (Data, 2002–2012, satellite source – GHRSST L4 Remote Sensing System MW IR OI SST and GHRSST L4 AVHRR AMSR OI; http://poet.jpl.nasa.gov/). Water temperature at the time of collection, was 0.5–1.1 °C. All gamete preparations and experiments were conducted using fresh 1 μm filtered (Millipore) seawater. The filtered seawater (FSW) in 6 analysed samples had a salinity of 33–34 (mean = 34.5 ± 0.15 PSU), dissolved oxygen (DO) > 90%, total pH (pH_T) = 8.00 ± 0.003, total alkalinity