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Impacts of ocean acidification on sperm develop with exposure time for a polychaete with long lived sperm

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ABSTRACT

The majority of marine invertebrate species release eggs and sperm into seawater for external fertilisation. Seawater conditions are currently changing at an unprecedented rate as a consequence of ocean acidification (OA). Sperm are thought to be particularly vulnerable to these changes and may be exposed to external environmental conditions for variable periods of time between spawning and fertilisation. Here, we undertook a mechanistic investigation of sperm swimming performance in the coastal polychaete *Arenicola marina* during an extended exposure to OA conditions (pH_{NBS} 7.77, 1000 μatm pCO_2). We found that key fitness-related aspects of sperm functioning declined faster under OA conditions i.e. impacts became apparent with exposure time. Sperm swimming speed (VCL), the number of motile sperm and sperm path linearity all dropped significantly after 4 h under OA conditions whilst remaining constant under ambient conditions at this time point. Our results highlight the importance of sperm exposure duration in ocean acidification experiments and may help towards explaining species specific differences in response.

1. Introduction

The vast majority of marine invertebrate species reproduce via the ancestral mode; external fertilisation (Giese and Kanatani, 1987; Wray, 1995), whereby eggs and sperm are released into the seawater column to fertilise. Marine external fertilisers may be vulnerable to environmental stressors such as climate change and pollutants, as their reproduction relies upon the successful meeting of gametes in the seawater column (Lewis and Ford, 2012). The gametic phase is often the most sensitive stage in an organism's life (Marshall, 2006; Pineda et al., 2012): gametes face all the challenges of environmental stressors at a small size and with only limited protective mechanisms. Sperm may be particularly susceptible as they are presumed to have no actively transcribing nuclear genes or biochemistry limiting their ability to respond to adverse environmental conditions through regulation. They also have little capacity to repair DNA damage and lack antioxidant defences or cellular repair mechanisms (Aitken et al., 2004). The environment sperm experience can have far-reaching consequences and influence both fertilisation success and offspring developmental success (Lewis and Galloway, 2009; Ritchie and Marshall, 2013). Motility is fundamental to a sperm's ability to function. Whilst sperm can be transported and dispersed rapidly in seawater by the action of currents, active swimming is likely to be required to bring sperm into direct contact with the surface of an egg (Kamp et al., 1996). Hence, any environmental stressor that perturbs sperm motility could severely hamper sperm function.

The sperm of broadcast spawning marine invertebrates can have wide ranging longevity depending on species, remaining fertilisation competent for just a few minutes (Pennington, 1985) after release into seawater up to several days (Williams and Bentley, 2002). For example, whilst many sea urchins have a sperm longevity of 1–2 h, Ohtake et al. (1996) report for the urchin *Hemicentrotus pulcherriumus* that after 12 h in seawater, 90% of sperm were still motile and after 20 h, 40% were still motile. Suquet et al. (2010) found that sperm from the oyster *Crassostrea gigas* were still motile after 20 h (supported by pers. obs) and Powell et al. (2001) showed that in Antarctic limpets sperm longevity was >90 h

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in *Laternula elliptica*, and ~65 h in *Nacella concinna*. Sperm longevity is also known to vary significantly within a single ejaculate and has been shown to influence offspring quality (Crean et al., 2012). Hence, marine invertebrate sperm may experience an extended exposure to environmental conditions before fusing with an egg. This differs from most species of marine fish, which tend to spawn in short bursts in close proximity to one another, favouring faster but shorter lived sperm lasting just seconds (Lahnsteiner and Patzner, 1998) to tens of minutes (Cosson et al., 2008).

The difficulties in predicting the timing and location of spawning events for most externally fertilising marine invertebrates means they have rarely been studied *in situ,* hence there is very little field data estimating the seawater contact time for successful sperm. Most of our knowledge on fertilisation dynamics comes from laboratory and theoretical studies. Early work by Pennington (1985) and Denny and Shibata (1989) highlighted the potential for rapid sperm dilution below fertilisable concentrations in seawater, hence it was widely accepted that most fertilisation takes place within seconds or minutes of sperm release (Levitan and Petersen, 1995). However, recent studies have challenged this view and proposed several factors that can extend the period before sperm are diluted below a threshold concentration for fertilisation (Yund, 2000). These include the release of sperm in very large numbers (Babcock and Mundy, 1992; Babcock et al., 1994) or in viscous fluids (Thomas, 1994), which act to slow sperm dispersal. Spawning during calm periods (Serrao et al., 1996), with reduced water motion or into semi-enclosed bodies of water such as rock pools or surge channels (Denny et al., 1992) can also prevent rapid sperm dilution (Yund, 2000). In certain cases, such as when gamete collisions are rare, the period of sperm fertilising ability (termed longevity) influences fertilisation success (Levitan, 2006).

The seawater physico-chemical conditions into which these sperm are released are currently changing at a rate unprecedented in the geological record (Hönisch et al., 2012), as increasing atmospheric carbon dioxide (CO_2) levels drive elevated sea surface temperatures and decreased oceanic pH and carbonate saturation states (termed ocean acidification: OA). The pH of surface seawater has already decreased by 0.1 pH unit since industrialisation (Stocker et al., 2013), with further reductions of 0.22–0.35 pH units projected by the end of the century unless emissions are dramatically cut (Bopp et al., 2013). The majority of studies investigating the effects of OA conditions on reproduction in broadcast spawners look at fertilisation success rather than measuring sperm function directly. These studies have shown that fertilisation can be negatively impacted by OA conditions for species across broad taxonomic groups including Cnidaria (Albright and Mason, 2013; Albright et al., 2010), Echinodermata (Gonzalez-Bernat et al., 2013b), Mollusca (Barros et al., 2013; Parker et al., 2009) and Polychaeta (Campbell et al., 2014). However, other studies have reported external fertilisation to be tolerant of experimental OA (Byrne et al., 2009; Chua et al., 2013; Havenhand and Schlegel, 2009; Ho et al., 2013; Martin et al., 2011) including a suite of marine invertebrates from South-East Australia (Byrne et al., 2010). These contradictory responses, reported for the same species by different research groups, may well be a result of population-specific gamete sensitivities that may be driven by adaptation to local environmental conditions (Kapsenberg et al., 2017), or they may simply result from different methodologies employed by studies. When sperm swimming has been directly examined, rather than using fertilisation success as the end point, most investigations observed significant reductions in sperm swimming speed and/or the percentage of motile sperm in an ejaculate for at least one OA treatment level (Havenhand et al., 2008; Morita et al., 2010; Schlegel et al., 2012; Vihtakari et al., 2013). However, again there appear to be differences between species with some studies observing no effect for either swimming speed or percent motility endpoints (Havenhand and Schlegel, 2009; Nakamura and Morita, 2012; Sung et al., 2014) and one found that sperm swimming performance was enhanced under OA conditions (Caldwell et al., 2011) adding to mounting evidence of species-specific sperm responses (Frieder, 2014).

Sperm concentration is arguably the single greatest influence on external fertilisation success (Levitan, 1991; Levitan et al., 1991). When studies investigated a range of sperm concentrations, several found the influence of OA conditions on fertilisation was sperm concentration-dependent (Ericson et al., 2010; Gonzalez-Bernat et al., 2013a) with stronger OA effects at lower, and potentially more environmentally relevant sperm concentrations (Levitan and Petersen, 1995). Fertilisation assays often test a single sperm concentration and biological effects could be missed by not investigating the full range of field-relevant sperm-to-egg ratios. There is also a tendency for assays to use relatively high sperm concentrations that can result in nearly 100% fertilisation success across a range of experimental treatments (for example see; Martin et al., 2011). This prohibits the identification of positive effects, and could mask the observation of more subtle, but biologically relevant, influences on fertilisation through sheer sperm numbers. Saturating sperm concentrations are unlikely to be ecologically relevant to most populations, as although field data is rare, when present it reveals that the percentage of a female's eggs fertilised is often much less than 100% (Levitan, 1998; Williams et al., 1997). Sperm motility is a key fitness-related aspect of sperm function and we propose there is a need for more ecologically relevant studies to look at OA impacts directly on sperm function.

One of the logistical constraints to conducting OA-sperm exposures is that high sperm respiration rates rapidly alter the seawater pCO_2 levels modifying the exposure conditions. In order to overcome this, we developed a novel technique that allowed us to control the pH and oxygen content of sperm incubations, without mechanical disruption from direct air bubbling. We constructed self-contained incubation chambers from dialysis membranes designed to retain the sperm cells inside the chamber, but allow the rapid exchange of oxygen and carbon dioxide between the chamber and a large seawater reservoir where the carbonate chemistry was monitored and manipulated by bubbling. Using this technique, we undertook a mechanistic exploration of sperm performance over 8 h under simulated OA conditions in the coastal polychaete *Arenicola marina* without disruption from direct bubbling. Found in intertidal sediments across Northern Europe, *A. marina* is a keystone species and ecosystem engineer (Volkenborn et al., 2007). It plays important roles irrigating and bioturbating the sediment, and as a secondary producer and prey species for fish and wading birds. *A. marina* has an unusual reproductive strategy where interaction between sperm and eggs may take place several hours after spawning and dilution in seawater (Williams and Bentley, 2002). Hence, in a future high CO_2 ocean this may result in a prolonged sperm exposure to OA conditions in this species. Our aims were to (1) establish the influence of OA conditions on sperm motility in *A. marina* over time and (2) to investigate potential mechanisms underlying any observed changes in sperm swimming behaviour by monitoring sperm oxygen consumption, ATP content and viability over time.

2. Materials and methods

2.1. Animals and experimental set-up

Ripe worms collected from Mothecombe beach, Devon, UK (50°31′23 N, -3°94′58 W) were acclimatised to laboratory conditions (14.5 °C, pH_{NBS} 8.10) for at least seven days prior to spawning.