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Reproductive toxicity of the water accommodated fraction (WAF) of crude oil in the polychaetes *Arenicola marina* (L.) and *Nereis virens* (Sars)

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ABSTRACT

Accidental pollution incidents are common in the marine environment and are often caused by oil-related activities. Here the potential of such an incident to disrupt reproduction in two polychaete species is investigated, using an environmentally relevant preparation of weathered Forties crude oil, i.e. the water accommodated fraction (WAF). Oocytes were collected and exposed to three concentrations of WAF for 1 h prior to the addition of sperm, so that fertilization took place under exposure conditions. Fertilization success was significantly reduced in both species by an exposure to WAF concentrations equivalent to 0.38 mg L^{-1} PAHs, to just 26.8% in *Arenicola marina* compared to 76% in *Nereis virens*. The effects of WAF exposure on fertilization were greatly enhanced at lower sperm concentrations in *N. virens*, with a complete lack of fertilization reactions observed at sperm concentrations of 10^3 sperm per mL. We therefore suggest a mechanism of toxicity related to sperm swimming behaviour, resulting in reduced sperm:egg collision rates. WAF was found to reduce post-fertilization development rates and have teratogenic effects on early embryonic stages in both species, which exhibited abnormal cleavage patterns and high levels of fluctuating asymmetry. These results illustrate how the presence of crude oil in its soluble form in seawater at the time of a spawning event for either *A. marina* or *N. virens* could impact on fertilization success with implications for the fertilization ecology of these free spawning marine invertebrates.

1. Introduction

Crude oil enters the marine environment from a number of natural and anthropogenic sources, though both chronic pollution and accidental spills. The toxicological effects of acute exposures to oil pollution and polycyclic aromatic hydrocarbons (PAHs) on the health of aquatic organisms have received much attention (Bonsdorff et al., 1990; Bejarano et al., 2006), however the longer-term implications and population-level effects of such exposures are still poorly understood. Crude oil is highly unstable in the marine environment, quickly loosing the volatile fractions through weathering (Payne et al., 1992; Smith et al., 2006) and becoming both photo- and biodegraded. Prolonged turbulent mixing by wind and currents results in the release of a water accommodated (or soluble) fraction (WAF) mainly composed of naphthalene (Rossi and Anderson, 1976; Wolfe et al., 1998; Barron et al., 1999; Pollino and Holdway, 2002). This water-soluble fraction has been shown to be highly toxic to the embryos of fish (e.g. Middaugh and Whiting, 1995; Pollino and Holdway, 2002; Couillard et al., 2005); starfish (Davis et al., 1981) and crustaceans (e.g. Cucci and Epifanio, 1979; Johns and Pechenik, 1980) from pristine environments.

Polychaetes tend to form the dominant sediment dwelling fauna of most mud flats, estuaries and sheltered sandy shores, and so are often found in areas of chronic oil pollution or areas affected by acute spills. There is evidence to suggest certain polychaete species are particularly tolerant to PAHs (De Boeck and Kirsch-Volders, 1998; Lewis and Galloway, 2008). *Nereis virens* in particular is often found in sediments affected by chronic PAH contamination (Lewis and Galloway, 2008) and experimental field studies have shown some polychaetes are able to recruit soft sediments heavily contaminated with hydrocarbons in large numbers (Stark et al., 2003). Little is currently known, however, regarding any impacts of oil pollution on their fertilization or developmental biology.

WAF is the oil component most likely to have any effect on reproduction in free spawning sediment dwelling marine invertebrates during any spill incident as oocytes and sperm, and subsequently developing embryos and larval stages are released freely in the water column. The two polychaetes used in this investigation differ slightly from a typical broadcast spawning strategy in that their oocytes are spawned in the female burrow (in the case of *Arenicola marina*) or in shallow pools at low tide (*N. virens*), hence the exposure routes for oocytes will differ slightly from a true broadcast spawning species and are likely to be highly complex. Sperm are

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released freely into the incoming tide by both species, which are then drawn into the female burrow through irrigation behaviour of female *A. marina* or washed into the shallow pools containing the oocytes of *N. virens*, enabling fertilization to take place. Exposure to spilled oil contamination may, therefore, also occur via direct contact to stranded oil bound to intertidal sediments, however this would be complex and difficult to mimic experimentally. Hence for the purposes of this investigation we aim to focus on the soluble WAF components of the oil in which the sperm would be present and in which the fertilization reaction would therefore occur.

Invertebrate oocytes contain a significant amount of vitellin (yolk) which is a heavy (420,000 Da) lipoglycoprotein (Fischer and Schmitz, 1981), and therefore are highly likely to readily sequester hydrophobic PAHs. Most studies looking at PAH affects on sperm (mainly human and vertebrate studies) relate to metabolite-induced DNA damage (e.g. Gaspari et al., 2003; Hsu et al., 2006), however naphthalene and the other smaller water-soluble PAHs found in WAF are not widely considered to have genotoxic properties (reviewed by Schreiner, 2003). There have been a number of studies describing reduced fertilization rates in a number of broadcast spawning coral species exposed to petroleum-based extracts (e.g. Negri and Heyward, 2000; Harrison, 1994); however, the precise mechanisms by which WAF disrupts external fertilization have not been widely studied in marine invertebrates, with the majority of studies focusing on embryo and larval development.

The majority of larger marine invertebrate species, including *A. marina* and *N. virens*, reproduce by releasing their eggs and/or sperm into the water column so that fertilization takes place externally. These spawning episodes can be highly seasonal, with offspring production often confined to just 1–2 weeks of the year (e.g. Caspers, 1984; Goerke, 1984; Olive et al., 2000; Watson et al., 2000; Guest et al., 2005). The potential risks of such a highly confined spawning period being disrupted were illustrated in November 1981, when mass spawning on the Great Barrier Reef coincided with heavy rainfall. Propagules on the surface were killed, thereby destroying the whole reproductive effort of those corals for that year (Harrison et al., 1984). Similar catastrophic reproductive failure was described for *Mytilus edulis* when spawning coincided with a brown tide in Rhode Island (Tracey, 1988). Fertilization and early post-fertilization development are widely considered to be the most sensitive life history stages to disruption through disturbances such as temperature change or the presence of environmental pollutants (Andronikov, 1975; His et al., 1999). Successful fertilization is a critical step in the life history of a species, but particularly so for broadcast and free spawning marine invertebrate (Pennington, 1985), where sperm is often limiting and fertilization success is rarely 100% even under optimal conditions of temperature and water quality (Levitan, 1995). Population dynamics in many free spawning marine invertebrates is thought to be sperm limited (e.g. Denny and Shibata, 1989; Levitan, 1995; Yund, 2000), particularly where population density is low. Actual field data on sperm limitation and fertilization dynamics for free spawning marine invertebrates are sparse but a few studies have shown a proportion of oocytes may remain unfertilized (Levitan and Petersen, 1995; Williams, 1998; Bode and Marshall, 2007). Any additional effect of a pollutant on reduced fertilization success may therefore have significant implications for population dynamics.

In this study, the effects of an environmentally relevant preparation of weathered Forties crude oil, i.e. the WAF on reproduction and development in two polychaetes are investigated. The lugworm (*A. marina*) and the king ragworm (*N. virens*) are ecologically important benthic polychaetes, with highly seasonal epidemic spawning (Olive et al., 2000; Watson et al., 2000). Both of these species are found in areas regularly at risk from oil-related pollution events, hence it is essential that we understand the risks to the population resulting from gamete and larval exposure. Spawning episodes for *A. marina* are typically only 3–4 days long (Watson et al., 2000), which may exacerbate considerably the potential effects on a population's reproductive success. Less is known for *N. virens* but some parts of a population also seem to spawn over very short periods of a few days. Effects of WAF generated from weathered Forties crude oil on fertilization success and early post-fertilization for these two species are therefore determined via a series of laboratory exposures and the developmental stability of resulting larvae is determined using a fluctuating asymmetry (FA) bioassay.

2. Materials and methods

2.1. Collection and maintenance of animals

Mature *A. marina* specimens were collected from the beach at Mothercombe estuary, South Devon (50°18′41″ N, 3°56′45″ W), during October–November 2006. This site is relatively free from contamination according to Environment Agency (2007) data. Individual animals were collected according to the methods of Lewis et al. (2002) and returned to the laboratory where they were checked for maturity, then stored at 12 °C in individual containers in filtered (0.2 μm) seawater (FSW). Animals were maintained in 10-L glass aquarium tanks in well-aerated FSW for 2 days post-digging to allow their gut contents to be voided.

Adult *N. virens* were collected from the muddy shore at Torpoint, in the Tamar estuary, Cornwall (50°22′14″ N, 4°11′44″ W), during February 2006 and 2007. Large, mature specimens were collected by carefully digging with flat pronged forks and then removing the animals by hand. Specimens were returned to the laboratory and stored at 12 °C in individual containers in FSW. Sex and maturity of specimens was checked on return to the laboratory by the examination of a coelomic sample collected with a 1-mL syringe fitted with a 21-G hypodermic needle inserted between the segments in the posterior of the worm. Animals were maintained in individual glass aquarium tanks containers in well-aerated FSW until use.

2.2. Weathering of crude oil and production of WAF

Exposure solutions containing the WAF of crude oil were prepared under controlled temperature conditions (at 12 °C) according to a previously developed method (Scarlett et al., 2007; Smith et al., 2006). This method for generating WAF has been robustly tested and demonstrated to generate highly reproducible WAF in terms of its chemical composition, and the changes in chemical composition that occur over time under a number of conditions has been thoroughly analyzed and described (Smith et al., 2006). Fresh Forties crude oil was artificially weathered to simulate losses of the volatile (e.g. benzene, toluene, ethylbenzene and xylenes, BTEX) components of the crude oil (representing approximately 20% of the total hydrocarbon content) that would typically occur during the first 2–3 h at sea following an oil spill. Twenty-five millilitres of this weathered Forties crude oil was then slowly vortex mixed with 2475 mL of 36 psu filtered (0.2 μm) seawater at a ratio of 1:99 for 24 h, and then left to re-equilibrate for 1 h. After this mixing the clear solution of the water available fraction (WAF) was carefully siphoned off under low nitrogen pressure. Since WAF should contain the soluble components of the oil with minimal presence of droplets, the number of particles present in various size categories counted using a Beckman Z2 Coulter Particle Count and Size Analyser, and compared with that of seawater. WAFs were considered acceptable with particle counts <5× that of seawater (which represents twice the standard deviation observed for natural seawater