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Increased tolerance to oil exposure by the cosmopolitan marine copepod *Acartia tonsa*

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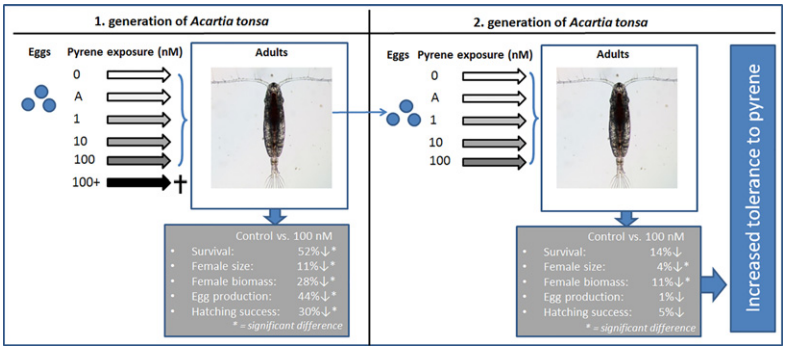
HIGHLIGHTS

- New knowledge on the tolerance of marine organisms to oil exposure
- The cosmopolitan copepod *Acartia tonsa* was exposed to pyrene for two generations.
- Pyrene (100 nM) reduced survival, grazing and egg production in the 1st generation.
- Survival, egg production and hatching success were recovered in the 2nd generation.
- Second generation of *Acartia tonsa* showed an increased tolerance to pyrene exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Oil contamination is an environmental hazard to marine ecosystems, but marine organism tolerance to oil after many generations of exposure remains poorly known. We studied the effects of transgenerational oil exposure on fitness-related traits in a cosmopolitan neritic copepod, *Acartia tonsa*. Copepods were exposed to an oil compound, the PAH pyrene, at concentrations of 1, 10, 100 and 100+ (the saturated pyrene concentration in seawater) nM over two generations and measured survival, sex ratio, size at maturity, grazing rate and reproductive success. Exposure to the pyrene concentration of 100+ nM resulted in 100% mortality before adulthood in the first generation. At the pyrene concentration of 100 nM, pyrene reduced grazing rate, increased mortality, reduced the size of females and caused lower egg production and hatching success. Importantly, we found strong evidence for increased tolerance to pyrene exposure in the second generation: the reduction in size at maturity of females was less pronounced in the second generation and survival, egg production and hatching success were recovered to control levels in the second generation. The increased tolerance of copepods to oil contamination may dampen the direct ecological consequences of a coastal oil spill, but it raises the concern whether a larger fraction of oil components accumulated in survived copepods, may be transferred up the food web.

1. Introduction

Oil pollution from shipping and oil exploitation is a major potential ecotoxicological hazard to marine ecosystems. It is well known that oil spills such as Exxon Valdez or Deepwater Horizon caused immediate catastrophes (Allan et al., 2012; Camilli et al., 2010; Joye, 2015; Peterson et al., 2003). For example, the Exxon Valdez oil spill killed between

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1000 and 2800 sea otters and approximately 250,000 seabirds (Peterson et al., 2003). Both shipping and oil exploitation have been predicted to increase in the near future to fulfill the increasing global energy demand, which may in turn increase exposure of marine organisms to crude oil worldwide (Barata et al., 2005; National Research Council, 2003; Nørregaard et al., 2014). However, very little is known about whether marine species, particularly those at the base of the food web such as copepods, will develop an increased tolerance when exposed to dissolved oil components e.g. polycyclic aromatic hydrocarbons – PAHs (Reddy et al., 2012; Samanta et al., 2002).

Oil spills from shipping, oil seepage and offshore oil exploitations introduce PAHs to the environment (Wolska et al., 2012) and PAHs are toxic to marine animals (Almeda et al., 2013; Barata et al., 2005; Incardona et al., 2012; Jensen et al., 2008). More than 20 different PAHs have been found in coastal water affected by oil spills and pyrene is one of the very common PAHs in crude oil (Reddy and Quinn, 2001; Reddy et al., 2012). Pyrene has relatively low toxicity to marine organisms compared to other PAHs (Barata et al., 2005). Furthermore, the toxic effect of PAHs in mixture has been shown to be additive (Barata et al., 2005), therefore the use of pyrene as a model PAH does not overestimate the toxicity of total PAHs (Jensen et al., 2008). The concentration of pyrene in coastal water varies from the trace level (Annammala et al., 2013) to approximately 2.3 μg L^{-1}, equal to approximately 12 nM (Reddy and Quinn, 2001), but the total PAH concentration can be up to 115 μg L^{-1}. The Deepwater Horizon oil spill released approximately 387 tons of pyrene to the Gulf of Mexico (Reddy et al., 2012), but there has not been given any clear information about the concentration in the seawater.

There is evidence that aquatic animals may develop a tolerance to contaminants (e.g., Bach and Dahllof, 2012; Klerks et al., 2011; Morgan et al., 2007; Ross et al., 2002) or toxins from phytoplankton (e.g., Colin and Dam, 2002, 2005; Dam, 2013). The multigenerational experiment is a powerful approach for detecting the development or expression of an increased tolerance to contaminants or toxins across generations (e.g. Carrera-Martinez et al., 2011; Colin and Dam, 2005; Romero-Lopez et al., 2012). Recent studies have stressed the need for multigenerational ecotoxicological studies to fully evaluate the effects of contaminants on the persistence of natural populations (Kimberly and Salice, 2015; Perrichon et al., 2015; Prud'homme et al., 2017). However, the potential for fast-growing marine copepods with short life cycles to develop an increased tolerance to PAHs across a transgenerational exposure has not been investigated.

In the coastal ecosystem, copepods play a key role as secondary producers transferring energy up through the food web to the fish stock (Kiørboe, 1998; Kwok et al., 2015). Any negative effects of oil exposure on copepods may therefore cascade through the pelagic food web with huge potential ecological and economic consequences. Copepods are commonly used as model species in ecotoxicological studies of coastal marine ecosystems (Kwok et al., 2015; Raisuddin et al., 2007). Exposure to PAHs is known to reduce growth and fecundity and increase mortality of marine copepods (Bellas and Thor, 2007; Hjorth and Nielsen, 2011; Grenvald et al., 2013).

In this study, we tested the hypothesis that the short life cycled copepod *A. tonsa* would develop an increased tolerance to pyrene exposure after a continuous two-generation exposure. Fitness-related traits such as mortality, size at maturity, sex ratio, grazing rate, egg production and hatching success were quantified. We chose *A. tonsa* due to it being one of the most abundant copepod species in nearshore marine environments globally (Chen and Hare, 2008; Chen and Hares, 2011; Cervetto et al., 1999; Paffenhöfer and Stearns, 1988; Pastorinho et al., 2003).

2. Materials and methods

2.1. Study species

Eggs of the copepod *Acartia tonsa* were obtained from a stock culture at the National Institute of Aquatic Resources, Technical University of Denmark. The culture of *A. tonsa* has been in the laboratory for >30 years and is reared in 513 L black polyethylene tanks (h × d = 150 × 66 cm) filled with ca. 450 L filtered seawater. Temperature is stable at around 16–18 °C (Støttrup et al., 1986) and salinity was kept stable at 32 ppt. The culture is provided with dim light which follows the natural diel cycle (see more details in Drillet et al., 2008a). They are fed ad libitum with the microalgae *Rhodomonas salina* (500 μg C L^{-1}) (Berggreen et al., 1988). The density of the adult copepods in the stock culture is maintained at approximately 100 individuals L^{-1} and the eggs are harvested three times a week (Støttrup et al., 1986). Depending on the temperature, it takes between 12 and 23 days for an egg to develop to the adult stage (Finiguerra et al., 2013) and the lifespans of adult males and females of *A. tonsa* vary from 10 to >40 days depending on food availability (Finiguerra et al., 2013; Kiørboe et al., 2015). Each female can lay approximately 25 eggs per day on average (Støttrup et al., 1986).

2.2. Filtered seawater and pyrene solutions

Seawater (salinity of 32 ppt) was filtered through a two-time 0.2 μm filtering system. Water was kept in the laboratory for 24 h prior to experimental use to stabilize the temperature. pH was 8–8.2 and the dissolved oxygen was approximately 7 mg L^{-1}.

Two stock solutions of 0.2 (stock 1) and 1 mM (stock 2) were made by dissolving pyrene powder (Sigma-Aldrich, purity > 99%) in absolute acetone. Both stock solutions were kept in amber glass bottles wrapped in aluminum foil to avoid photodegradation of pyrene. Stock solutions were kept at a room temperature of approximately 20 °C. The exposure solutions were daily prepared by diluting the stock solutions. Stock 1 was diluted 5000 and 500 times in filtered seawater to prepare the exposure solutions of 1 and 10 nM, respectively. Similarly, stock 2 was diluted 10,000 and 3333 times in filtered water to obtain the exposure solutions of 100 and 100+ nM, respectively. The concentration of acetone in the acetone control was 300 μl L^{-1}, which was equal to the acetone concentration in the highest pyrene exposure solution (100+ nM). The laboratory was kept dark during the experiment as the toxicity of pyrene substantially increases when exposed to sunlight (Pelletier et al., 1997).

2.3. Pyrene exposure experiment

To test whether copepods develop an increased tolerance to oil exposure a transgenerational experiment was conducted in which *A. tonsa* was exposed to six different exposure solutions: seawater control, acetone solvent control, 1, 10, 100 and 100+ (saturated pyrene concentration in seawater, Nørregaard et al., 2014) nM pyrene for two generations. The pyrene concentrations were chosen based on previous studies showing negative effects on the performance of marine copepods (Jensen et al., 2008; Grenvald et al., 2013; Nørregaard et al., 2014). The highest pyrene concentration in this study (100+ nM, equal to approximately 90 μg L^{-1}) was lower than the concentration of total PAHs, of 115 μg L^{-1} measured in seawater affected by oil spills (Reddy and Quinn, 2001). The measured pyrene concentrations in exposure solutions (based on pooled samples collected from 5 different bottles per concentration) were 0.7, 8, 164 and 457 nM when the medium was freshly renewed. After 24 h, the real exposure concentrations were 3, 7, 152 and 415 nM, respectively. An independent research group (Lovap, Belgium) performed all measurements of pyrene concentrations. The acetone control was included in the experiment as acetone was used as a solvent for pyrene. An acetone concentration of up to 900 μl L^{-1} (three times higher than the highest acetone concentration used in this experiment) has no effect on survival and fecal pellet production in another calanoid copepod, *Calanus finmarchicus* (see Appendix A). Each treatment had five replicates (a total of 30 experimental units for the first generation and 25 experimental units for the second generation).