



Differential survival and reproductive performance across three mitochondrial lineages in *Melita plumulosa* following naphthalene exposure



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HIGHLIGHTS

- We test the response of an invertebrate bioindicator to naphthalene exposure.
- Animals of different mitochondrial lineages demonstrated differential survivorship.
- Different mitochondrial lineages also showed differential reproductive performance.

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ABSTRACT

Populations subject to anthropogenic contaminants often display altered patterns of genetic variation, including decreased genetic variability. Selective pressures of contaminant exposure are also reflected in differential tolerance between genotypes. An industrial chemical spill in a major eastern Australian waterway in July 2006 resulted in altered patterns of genetic variability in a nearby population of the amphipod, *Melita plumulosa* for up to one year post-spill, despite the site being declared clean after 48 h. Here, we investigate the toxicant response of three mitochondrial lines naturally occurring at the impacted site by comparing survivorship and life-history trait variables following naphthalene exposure. Overall, *M. plumulosa* demonstrated differential survivorship between mitochondrial lines under exposure to high concentrations of naphthalene. In addition, we identified differential fecundity and frequencies of gravidity in female amphipods between the mitochondrial haplotypes examined. These findings suggest that the patterns of genetic variability previously identified may be linked with differential tolerance and/or reproductive performance between mitochondrial lineages.

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1. Introduction

Populations are subject to a variety of environmental stressors that exert selective pressures on their genetic structure, both natural and anthropogenic in origin. The impact of anthropogenic stressors such as toxicants and contaminants on the genetic structure of natural populations has come under increasing scrutiny as more light is shed on the effects and mechanisms of contaminants. In some cases, exposure to anthropogenic contaminants has been shown to increase genetic variability through increased mutation rates (e.g. Theodorakis and Shugart, 1997; Rinner et al., 2011). Conversely, other studies have shown contaminants to act as a selective pressure resulting in genetic depauperation of populations (e.g. Keklak et al., 1994; Cohen, 2002). Where contaminants

were shown to exert selective pressure on a population, differential survival between genotypes can also be demonstrated (Duan et al., 2001; Schizas et al., 2001). For example, three naturally occurring mitochondrial lineages in the copepod *Microarthridion littorale* demonstrated differential survivorship following pesticide exposure (Schizas et al., 2001). Contaminant exposure can also result in population changes at the organismal level by impacting life-history traits including reproductive performance (Clarke et al., 2009; Ringwood et al., 2009). Clarke et al. (2009) found that ramshorn snails exposed to treated sewage effluent were heavier and larger than unexposed animals, as well as being more fecund. The aim of this study was to determine whether different genetic lineages display differential tolerance and/or reproductive performance following toxicant exposure in the amphipod *Melita plumulosa*.

One group of chemicals commonly present in many anthropogenic contaminants is polycyclic aromatic hydrocarbons (PAHs). PAHs encompass a large class of toxic compounds comprised of

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two or more adjoined benzene rings, and are prevalent in many petrochemical and crude oil products. As such, PAHs are common throughout many aquatic environments as a result of industrial effluent discharge, petrochemical spills and petroleum refinery and combustion (Walker et al., 2001). Low molecular weight fractions such as naphthalene and naphthalene compounds are known to be acutely toxic to many aquatic species, although chronic toxicity is unlikely due to the volatile nature of these compounds (Wu et al., 2012). Naphthalene and naphthalene compounds readily diffuse through tissues such as gills and have been shown to bioaccumulate in various tissues in numerous aquatic invertebrate species (Laurén and Rice, 1985; Mhadhbi et al., 2010). Juvenile animals are particularly sensitive to naphthalene due to their smaller body size and higher diffusion rates (Aas et al., 2000). The reproductive performance of a population as well as other population and life-history traits have also been shown to be impacted by naphthalene exposure (Marquis et al., 2006; Krång, 2007; Pollino et al., 2009). In one study, sublethal exposure of crabs to naphthalene was shown to disrupt male pheromone reception resulting in decreased mate search behaviors (Krång, 2007). Another study on rainbow trout found that naphthalene exposure resulted in the disruption of sex steroid production leading to increased testosterone levels in both male and female fish, and thus an increase in male individuals throughout the population (Pollino et al., 2009).

The amphipod *M. plumulosa* is endemic to many estuarine environments along the eastern coast of Australia (Zeidler, 1989) and is currently being utilized as the major invertebrate indicator for monitoring the health of estuarine sediments. This invertebrate species is relatively abundant and simple to sample, as well as being amenable to laboratory culture (Hyne et al., 2005). Its employment as an indicator of estuarine health is due to its sensitivity to a range of contaminants including metals and PAHs in both aqueous and sediment-bound phases (King et al., 2005; Mann et al., 2009; Simpson and Spadaro, 2011). Juvenile animals in particular show greater sensitivity to contaminants than adult animals in both aqueous and sediment exposures (King et al., 2006).

In July 2006, an industrial toxicant spill occurred in the upper reaches of the Parramatta River in Sydney, Australia. This resulted in approximately 3000 L of a highly flammable tile spray containing an acrylate/methacrylate co-polymer and a variety of aromatic hydrocarbons in solvent naphtha to leech into surrounding waters and sediments (Land and Environment Court of NSW, 2008). A previous study found that the genetic structure of *M. plumulosa* at a site 1 km downstream of the spill were significantly impacted following the chemical spill (Chung et al., 2011). A significant increase in individuals harboring mitochondrial the cytochrome *c* oxidase subunit I (COI) defined haplotype 1 and a significant decrease in the level of genetic variability as determined by Tajima's *D* were found for up to one year post-spill, a pattern not found at other more distant localities along the Parramatta River (Chung et al., 2011).

We hypothesize that amphipods harboring different mitochondrial types will be differentially affected in their survival and/or reproductive performance in response to naphthalene exposure. Specifically, we hypothesize that amphipods harboring haplotype 1 which was found in excess following the spill are likely to have higher rates of survival or higher reproductive performance following exposure. To test this hypothesis, cultured amphipods harboring haplotypes 1, 3 and 8 naturally occurring at the site of the spill were subject to a pulse exposure of naphthalene. These exposure and recovery conditions are designed to mimic the conditions of the chemical spill of July 2006 for the purpose of examining possible molecular causes for the altered pattern of genetic variability identified previously (Chung et al., 2011). Although the specific chemical composition of the tile spray from the chemical spill is unknown, we chose to test the effect of naphthalene, as it makes

up much of the water soluble component of the tile spray and is known to be acutely toxic to aquatic organisms. Here, we identify differential survival between mitochondrial haplotypes following exposure to a relatively high concentration of naphthalene, and differences in fecundity following recovery from low concentration exposure. We also identify differences in the frequencies of female gravidity between mitochondrial lineages. These results may partially account for the patterns of genetic variability previously identified (Chung et al., 2011).

2. Materials and methods

2.1. Test organisms

To investigate possible molecular links between contaminant exposure and the altered patterns of genetic variability in amphipods impacted by the chemical spill of July 2006, we examined three mitochondrial haplotypes found at the impacted locality post-spill. Individuals harboring haplotype 1 were found to be in excess within the 12 months immediately post-spill, whereas the second most common haplotype at Duck River (haplotype 3) was largely absent (Chung et al., 2011). We also chose to test amphipods harboring haplotype 8 as a control. It is a rare haplotype identified in samples collected one year after the chemical spill (Chung et al., 2011). Haplotypes 1 and 3 differ by five synonymous base changes; haplotype 8 is an intermediate haplotype between haplotypes 1 and 3, and differs from haplotype 1 by three synonymous base changes and from haplotype 3 by two synonymous base changes.

Adult *M. plumulosa* were sampled at the Duck River site (33°49'28.74"S, 151°03'05.18"E) in Sydney, Australia in December 2008. This locality corresponds to the site previously found to be impacted by the chemical spill of July 2006, and is where amphipods harboring mitochondrial haplotypes 1 and 3 are most common (Land and Environment Court of NSW, 2008; Chung et al., 2011). The Duck River site is subject to chronic contamination as it is located in a heavily industrialized region and lies immediately downstream of a petrochemical refinery. *Melita plumulosa* were also sampled from a single locality from the George Rivers at Davy Robinson Point (33°55'50.10"S, 150°58'07.70"E) in Sydney, Australia, where amphipods harboring haplotype 8 are common (unpublished data). The Davy Robinson Point locality adjoins a park along the middle reaches of the Georges River and is considered relatively uncontaminated.

Cultures from single female and male pairs (isofemale cultures) were established from sampled animals, with founding animals removed after 4 weeks to minimize environmental carry-over effects (Hercus and Hoffmann, 2000; Hyne et al., 2005). A 630 bp region of the mitochondrial COI was then sequenced according to Chung et al. (2008) to identify the three haplotypes of interest. A single culture of each haplotype was then retained and maintained under standard clean laboratory conditions (Hyne et al., 2005) for one year prior to the establishment of cyto-nuclear introgression lines.

To investigate the relationship between mitochondrial haplotype and toxicant response, cyto-nuclear introgression lines (i.e. known mitochondrial lines against various nuclear backgrounds) were established using the three mitochondrial lines of interest. This strategy is expected to mimic the naturally occurring population if there is random mating between females harboring distinct mitochondrial types (Ballard et al., 2002) and enables a more rigorous examination of the effect of mitochondrial type on toxicant response. In January 2010, 5–15 females from each mitochondrial line were placed into fresh culture media with 5–15 males from each line in each of the nine possible combinations. Four replicates per line were established for each cross and subsequently main-

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