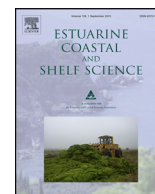




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# The impact of historic isolation on the population biogeography of *Melita plumulosa* (Crustacea: Melitidae) in eastern Australia

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## ABSTRACT

The genetic structure of populations is impacted by environmental factors of both natural and anthropogenic origin. These factors can affect dispersion, gene flow and selective pressures. We test whether natural environmental factors or anthropogenic factors influence the genetic structure of the amphipod, *Melita plumulosa* (Crustacea: Melitidae), which serves as an indicator of environmental health in estuaries along eastern Australia. Sequence data from one mitochondrial and two nuclear loci were collected and analyzed from eight geographically distinct populations spanning the known distribution of this species. We identified two major clades corresponding to the east and south coasts of Australia, and populations also largely grouped according to geography within each clade. Population differentiation indicated all sampling localities to be distinct from one another and sequence divergences suggested ancient divergence, with the deepest genetic divergences between the eastern and southern populations. Reproductive compatibility did not indicate cryptic speciation between populations. Sequence divergence and population differentiation suggest historic geographic isolation dating back to the Pleistocene to have influenced the population biogeography of *M. plumulosa*.

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## 1. Introduction

Genetic structure and diversity impacts numerous aspects of population biology, including persistence and the ability to respond to changes in environmental pressures (Johannesson and André, 2006; Hughes, 2007). Aquatic and marine organisms with limited dispersal capabilities and which are restricted to the water throughout their lifecycle are particularly susceptible to population subdivision through reproductive isolation or local selection/adaptation (Meyran et al., 1997; Mathews, 2006). This is most evident in marginal populations of widely distributed species, particularly among Crustacea (see Knowlton, 2000; Bilton et al., 2002 for reviews).

The genetic structure of populations is impacted by environmental factors of both natural and anthropogenic origin. In many aquatic and coastal environments, natural factors such as historic geological and climatic shifts cause periods of flood and drought that impact the dispersal and gene flow between populations

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(Hewitt, 1996, 2004; Carini and Hughes, 2006). Numerous aquatic invertebrate species inhabiting Australia's coastal waterways show evidence of genetic subdivision resulting from historical geographic isolation (Williams and Benzie, 1997; Carini and Hughes, 2004; Haig et al., 2010). In freshwater crayfish, patterns of genetic diversity reflect population subdivision dating back to the Pleistocene with low contemporary gene flow along the north-eastern Australian coastline (Bentley et al., 2010). Crayfish populations were found to display strong latitudinal structure at both mitochondrial and nuclear markers with little contemporary mixing between clades, as well as limited haplotype sharing between sampling localities within a clade (Bentley et al., 2010).

Alternatively, anthropogenic activities may be a primary force shaping the genetic diversity and population substructure. Exposure to anthropogenic contaminants is known to impact the genetic structure of populations by increasing mutation rates, or decreasing genetic diversity through selection (reviewed in Bickham, 2011). This is particularly evident in populations inhabiting aquatic environments (Bickham et al., 2000; Jones, 2010), which can accumulate high levels of chemical contaminants from urban run-off, industrial discharge and pesticide use (Cohen, 2002; Rinner et al., 2011). A study of the allozymatic diversity among populations of the sandhopper, *Talitrus saltator*, along the Tyrrhenian coast of Italy

found a direct correlation between genetic diversity and contamination, with amphipods from sites with the highest mercury concentrations displaying the lowest genetic diversity (Ungherese et al., 2010). Common contaminants identified in sediments in the major ports along eastern Australia are dissolved and sediment-bound organic contaminants including polycyclic aromatic hydrocarbons and metals (Birch, 2000; Roach et al., 2009).

In Australia *Melita plumulosa* is an endemic epibenthic amphipod of estuarine sediments. Historically, it has been found in many waterways along the east Australian coast between Brisbane in the north and Melbourne in the south, although the full geographic range of this species remains undetermined (Lowry et al., 2000). This crustacean is motile along the intertidal zone, and is found at the interface between water and sediment beneath rocks or shell-grit. It tolerates a wide range of sediment particle sizes (silt to gravel), salinities (freshwater to seawater), and temperatures (Hyne et al., 2005; King et al., 2006). *M. plumulosa* display sensitivity to a range of sediment-bound metals commonly found in industrial contaminants (King et al., 2006), and currently serves as the major invertebrate indicator of the health of estuarine sediments (Simpson et al., 2005; Simpson and Spadaro, 2011). A previous study identified differences in the genetic structure as well as phenotypic traits of *M. plumulosa* populations from two geographically distinct waterways with different contaminant profiles not evident among populations within the same waterway (Chung et al., 2008). Furthermore, amphipods exposed to chemical spill displayed a different pattern of genetic diversity from unimpacted populations within the same waterway (Chung et al., 2011).

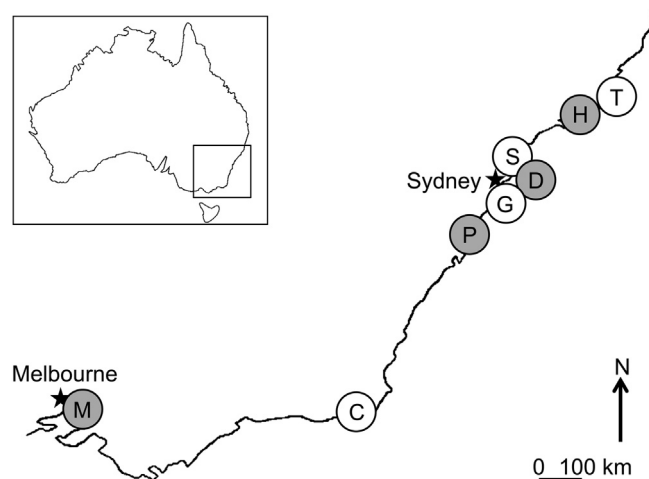
The goal of this study was to examine the genetic structure of the amphipod *Melita plumulosa* (Crustacea: Melitidae) as part of a larger project that critically evaluates the sensitivity and suitability of this species as an indicator of environmental health in estuaries along eastern Australia. We examined two hypotheses to explain the biogeographic patterns of *M. plumulosa* in eastern Australia. First, if historic environmental processes such as geographic isolation have been the predominant force shaping genetic structure, we hypothesized *M. plumulosa* populations will exhibit patterns of genetic diversity correlated with the time since the populations diverged, unless strong selection has been operating on the lineages. Second, we hypothesized patterns of genetic diversity to be strongly correlated with the presence of specific contaminants rather than geographic locality, with little evidence of substructure between uncontaminated populations.

In this study we characterized the patterns of genetic subdivision in *Melita plumulosa*, at one mitochondrial and two nuclear loci, at sites along the south-eastern coastline of Australia with varying levels of industrial contamination. We determined the environmental factors shaping the genetic structure of this broadly distributed bioindicator species. Analysis of population differentiation indicates strong genetic subdivision concordant with geography, but no distinct correlation with contaminant exposure.

## 2. Materials and methods

### 2.1. Sampling

*Melita plumulosa* were collected from late September to early December of 2009 from a single locality in each of eight geographically distinct river systems along the south-eastern coast of Australia (Fig. 1). Previously, it was established that genetic subdivision was not evident between populations sampled along the same river system suggesting panmixia within waterways, whereas populations between uncontaminated and contaminated waterways showed evidence of genetic structure (Chung et al., 2008).



**Fig. 1.** Sampling sites chosen along the south-eastern coast of Australia. The eastern localities were Twelve Mile Creek (T), Throsby Creek (H), Seymours Creek (S), Duck River (D), Georges River (G), and Port Kembla (P), and the southern localities were Cann River (C), and Moonee Ponds Creek (M). White numbered circles represent remote/uncontaminated sites and shaded circles represent sites of anthropogenic contamination.

Two major criteria were considered when sampling localities were selected in order to address the hypotheses proposed in this study. 1) To determine whether patterns of genetic variation were correlated with historic geographic isolation, we sampled populations across the known distribution of this species. 2) To determine whether patterns of genetic variation were correlated with the presence of anthropogenic contaminants, the eight river systems examined were divided into four pairs of waterways predicted as uncontaminated and industrially contaminated based on the presence of industrial activity.

From north to south the eight waterways sampled were: Twelve Mile Creek, Throsby Creek, Seymours Creek, Duck River, Georges River, Port Kembla, Cann River and Moonee Ponds Creek (Fig. 1). Twelve Mile Creek is a remote waterway near Swan Bay that enters into Port Stephens on the mid-north coast of New South Wales. Throsby Creek is a small creek that enters into the Hunter River in the heavily industrialized Newcastle region north of Sydney. Seymours Creek is a small waterway near the mouth of the Hawkesbury River north of Sydney. Duck River is a tributary of the Parramatta River, the major waterway flowing into Sydney Harbour, and is surrounded by industry including petrochemical facilities. Georges River is a major waterway in Sydney that enters into Botany Bay. Tom Thumb Creek in Port Kembla is a small creek in the heart of a heavily industrialized area in Wollongong, south of Sydney. Cann River is situated within Croajingolong National Park in south-eastern Victoria and is blocked by a transient sandbar at the mouth of the river over the Austral winter. Moonee Ponds Creek enters into the Yarra River in the center of Melbourne and is surrounded by dense urbanization and industry.

The waterways predicted to be uncontaminated were Twelve Mile Creek, Seymours Creek, Georges River and Cann River; the waterways predicted to be subject to industrial contamination were Throsby Creek, Duck River, Port Kembla and Moonee Ponds Creek (Fig. 1). It was predicted that sediments from Throsby Creek, Duck River and Port Kembla would likely exhibit a similar chemical profile due to related industry. To test our prediction that four waterways were uncontaminated and four were industrially contaminated, water quality data and sediment samples were collected at each locality. Two measures of surface water quality – water salinity (measured using the Practical Salinity Scale) and

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