



# Sensitivity and response time of three common Antarctic marine copepods to metal exposure



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## HIGHLIGHTS

- First publication on the toxicity of copper and cadmium to Antarctic copepods.
- Proposes new standard toxicity test procedures for Antarctic copepods.
- Highlights delayed response and long exposures in toxicity tests with Antarctic biota.
- Increases knowledge on adverse effects of contaminants on Antarctic marine biota.
- Provides sensitivity data for Environmental Quality Guidelines for Antarctica.

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## ABSTRACT

Understanding the sensitivity of Antarctic marine organisms to metals is essential in order to manage environmental contamination risks. To date toxicity studies conducted on Antarctic marine species are limited. This study is the first to examine the acute effects of copper and cadmium on three common coastal Antarctic copepods: the calanoids *Paralabidocera antarctica* and *Stephos longipes*, and the cyclopoid *Oncaea curvata*. These copepods responded slowly to metal exposure (4–7 d) emphasising that the exposure period of 48–96 h commonly used in toxicity tests with temperate and tropical species is not appropriate for polar organisms. We found that a longer 7 d exposure period was the minimum duration appropriate for Antarctic copepods. Although sensitivity to metal exposure varied between species, copper was more toxic than cadmium in all three species. *P. antarctica* was the most sensitive with 7 d LC50 values for copper and cadmium of 20  $\mu\text{g L}^{-1}$  and 237  $\mu\text{g L}^{-1}$  respectively. Sensitivities to copper were similar for both *O. curvata* (LC50 = 64  $\mu\text{g L}^{-1}$ ) and *S. longipes* (LC50 = 56  $\mu\text{g L}^{-1}$ ), while *O. curvata* was more sensitive to cadmium (LC50 = 901  $\mu\text{g L}^{-1}$ ) than *S. longipes* (LC50 = 1250  $\mu\text{g L}^{-1}$ ). In comparison to copepods from lower latitudes, Antarctic copepods were more sensitive to copper and of similar sensitivity or less sensitive to cadmium. This study highlights the need for longer exposure periods in toxicity tests with slow responding Antarctic biota in order to generate relevant sensitivity data for inclusion in site-specific environmental quality guidelines for Antarctica.

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## 1. Introduction

Natural concentrations of metals in the Antarctic marine environment are generally low (Honda et al., 1987). However, elevated metal concentrations relative to natural background levels have been found in sediments, water and biota in coastal areas of Antarctica that are close to scientific research stations as a result of local human activities. In particular, sites adjacent to abandoned waste tips, sewage outfalls, and sites where fuel spills have

occurred report elevated levels of a range of contaminants (Deprez et al., 1999; Stark et al., 2006; Tin et al., 2009; Kennicutt II et al., 2010). Changes in benthic community composition and biomass have been observed in some of these sites as a result of elevated concentrations of bioavailable metals in the water and in sediments (Lenihan and Oliver, 1995; Stark et al., 2003; Stark, 2008). Contaminants enter Antarctic marine ecosystems either by direct input from waste discharges, or indirectly during the summer melt when frozen contaminated soil thaws, mobilising contaminants into the nearshore marine environment. Here they can be dispersed through the water column, accumulate in sediments and be transferred to biota (Cole et al., 2000; Snape et al., 2001; Stark et al., 2006).

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Copper and cadmium are common contaminants in Antarctica (Larner et al., 2006; Scouller et al., 2006). While copper is an essential metal required for many physiological functions in organisms, cadmium is a non-essential metal with no known beneficial effects. However, both metals accumulate in body tissues and are potentially toxic when they reach certain threshold concentrations, which are both species- and metal-specific. It is well documented that the toxicity of a metal to an organism is influenced by abiotic factors, for example temperature, salinity and the presence or absence of chelating agents. The degree of toxicity to the exposed organism is also influenced by its physiological, nutritional and reproductive state, the life history stage and its bioaccumulation strategy (Rainbow et al., 1990; Rainbow, 2007).

Toxicity tests of 48–96 h duration are widely used in lower latitudes to assess the tolerance and sensitivity of species to contaminants including metals. Such data forms the basis of environmental quality guidelines. Data on the sensitivity of polar organisms to metals is scarce hence it is not possible to determine whether environmental quality guidelines developed in lower latitudinal regions can be applied to the Antarctic marine environment. This information is essential in order to carry out appropriate risk assessments for human activities in Antarctica, to guide remediation activities, and to develop targets for remediation at contaminated sites.

It is well known that polar marine invertebrates have longer life cycles and slower metabolism and growth rates resulting from adaptations to stable low temperatures and to seasonality of sea ice cover and primary production (Clarke and Peck, 1990; Peck, 2002). As a result of these unique characteristics, the sensitivity of Antarctic marine biota and response time to metal exposure is likely to be different from those of related organisms from lower latitudes (Chapman and Riddle, 2005; Chapman et al., 2006). The need to consider longer exposure times in toxicity tests reflecting the life span and metabolism of Antarctic species has been highlighted in previous studies (King and Riddle, 2001; Payne et al., 2014).

The few studies available on polar amphipods, mysids, and echinoid larvae have reported a range of sensitivities to copper, cadmium, zinc and lead compared with temperate species (Chapman and McPherson, 1993; Duquesne et al., 2000; King and Riddle, 2001; Liess et al., 2001; Duquesne and Liess, 2003). Most of these studies have focused on the effects of metals on benthic invertebrates or their early life history stages, while organisms that live pelagically for their entire life cycle have received little attention.

Zooplankton is an important component in Antarctic marine ecosystems as key organisms within the food web and in the biogeochemical cycling of elements (Swadling et al., 1997). As planktonic organisms, they are likely to be impacted by exposure to contaminants in the aqueous phase in the water column and through the ingestion of contaminated food. The calanoid copepods *Paralabidocera antarctica* and *Stephos longipes* and the cyclopoid *Oncaea curvata* have a circumpolar distribution and are dominant species of the summer zooplankton community in coastal ice-free areas of East Antarctica (Tucker and Burton, 1990). Copepod occurrence is largely driven by temperature, salinity, sea ice dynamics and predation (Swadling et al., 2004). Both calanoid species are associated with the sea ice. *P. antarctica* is the most abundant planktonic species during the austral summer when the sea ice is present while *S. longipes* abundance peaks immediately after the sea ice breaks out around February. In comparison, *O. curvata* is considered a deep oceanic species that undergoes seasonal vertical migrations into coastal areas, with highest abundances occurring between February and May (Tucker and Burton, 1990; Swadling et al., 1997).

In this study we conducted acute toxicity tests with the three common copepods within the zooplankton community in coastal

East Antarctica. The aims of the study were to (i) determine and compare the sensitivities of *P. antarctica*, *O. curvata* and *S. longipes* to copper and cadmium and (ii) develop standard methods for toxicity assessments with Antarctic copepods that include relevant longer-term exposure periods. This is to our knowledge, the first published data on the sensitivity of Antarctic copepods to metal contamination.

## 2. Materials and methods

### 2.1. Collection and acclimation of copepods

Experiments were done at Davis Station, East Antarctica (68°34' 36"S, 77°58' 03"E) during the 2010–2011 austral summer. Copepods used in toxicity tests were separated from plankton samples collected at two sites in Prydz Bay (68°34'S, 77°57'E and 68°33'S, 77°55'E). Prior to the sea ice breaking out (from 14 December 2010 to 21 January 2011), plankton were sampled via a hole drilled through the sea ice that was 1.8–1.3 m thick, using a specially designed collapsible umbrella net (Kirkwood and Burton, 1987) lowered to 20 m depth. The net was 28 cm in diameter and 2 m long, and was fitted with a 200 µm mesh and a hard cod end. After the sea ice broke out from the shoreline on the 27 January 2011, both sites were accessible by small inflatable boats. The same net was towed vertically behind the boat to sample plankton. After collection, plankton samples were transported to the Davis Station laboratory in seawater in 2.5 l insulated containers and held in a temperature controlled room at  $-1 \pm 1$  °C until sorted to obtain copepods. Adult copepods were separated from the plankton samples and identified using taxonomic keys and species descriptions using a dissecting microscope (LEICA) at 40× magnification. They were then acclimatised to laboratory and test conditions in the temperature controlled room at  $-1 \pm 1$  °C for 24–48 h in 500 mL beakers containing seawater with continuous aeration and no additional food. Seawater used for acclimating copepods and as the diluent in tests was collected 20 m offshore from Davis Station, well away from any obvious contaminant inputs associated with station activities and was filtered to 1 µm (filtered seawater; FSW). After the acclimation period, only healthy and active individuals that showed normal swimming ability were retained and used in tests.

### 2.2. Preparation and analysis of metal test solutions

Stock solutions (500 mg L<sup>-1</sup>) of copper (CuSO<sub>4</sub>·5H<sub>2</sub>O) and cadmium (3CdSO<sub>4</sub>·8H<sub>2</sub>O) were prepared using FSW and were stored in 250 mL high density polyethylene bottles in a refrigerator at 4 °C. All glass and plastic ware used in tests was washed in 10% (v/v) nitric acid (HNO<sub>3</sub>) and rinsed three times with Milli-RO water before use. All reagents were analytical grade. Plastic vials (70 mL) containing 50 mL of the test solution were prepared using FSW and the stock solutions 24 h before the start of tests and stored in a constant temperature cabinet (CTC) set at  $0 \pm 1$  °C. For the two replicate tests with *P. antarctica* and *S. longipes* and the three replicate tests with *O. curvata*, nominal concentrations were 0, 25, 50, 75, 100 µg Cu L<sup>-1</sup> and 0, 250, 500, 750, 1000 µg Cd L<sup>-1</sup>. Four replicates per treatment were used in all tests except those with *P. antarctica* in which only two replicates per treatment were used.

Samples of test solutions (see Section 2.3) were passed through a 0.22 µm Millipore syringe filter and acidified with 1% ultra pure HNO<sub>3</sub>, then stored in a fridge at 4 °C until analysed. Metal concentrations were measured using a Varian Inductively-Coupled Plasma Optical Emission Spectrometer (ICP-OES). Detection limits for copper and cadmium were 3 and 2 µg L<sup>-1</sup> respectively.

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