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Toxicity of copper to three common subantarctic marine gastropods

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ABSTRACT

Investigating the impacts of contamination on high latitude ecosystems includes determining the sensitivity of key taxa to contaminants. Unique characteristics, arising from adaption to cold and stable temperatures has likely resulted in marine biota at the poles being particularly sensitive to contamination in comparison to related species at lower latitudes. We aimed to determine the sensitivity of three species of common and ecologically important subantarctic gastropods to copper. This is the first study to investigate the sensitivity of subantarctic marine gastropods to contamination. We determined sensitivity by exposing each species to a range of copper concentrations by establishing mortality and sublethal endpoints. Sensitivity to copper was highly species specific. *Laevilittorina caliginosa* was relatively tolerant, with no response at Cu concentrations up to 1488 µg/L following 7 d of exposure, while two species (*Cantharidus capillaceus coruscans* and *Macquariella hamiltoni*) were highly sensitive with 7 d Cu LC50 estimates of 33 µg/L and 78 µg/L respectively. In a global comparison of gastropod sensitivity data, these two species were highly sensitive to copper, highlighting the vulnerability of polar ecosystems to contamination.

1. Introduction

Sensitivity of marine biota to contamination is likely to vary latitudinally (Holan et al., 2016; King and Riddle, 2001; Marcus Zamora et al., 2015). The unique characteristics of polar marine biota, such as slow metabolisms, longer development times and life spans, and growth to larger sizes, are common among species adapted to cold and stable temperatures (Chapman and Riddle, 2005). These characteristics likely result in longer exposure times, slower uptake, but also slower detoxification of contaminants. Biota in polar regions are therefore predicted to be more sensitive to contaminants (Chapman and Riddle, 2005). Furthermore, these communities have slow ecological recovery rates after a contamination event (Chapman and Riddle, 2005). While toxicity test methods have been developed and are routinely used for tropical and temperate species, very few methods have been developed for high latitude species (King and Riddle, 2001). It has been determined however, that specific test conditions are required, such as longer test durations, in order to compare their sensitivity with that of analogous species from other regions (Holan et al., 2016; King and Riddle, 2001; Marcus Zamora et al., 2015; Payne et al., 2014). Several calls for specific water quality guidelines for polar areas have been made, due to these likely differences in sensitivities, as guidelines developed in lower latitudes, such as those for Australia and New Zealand (ANZECC/ARMCANZ, 2000), may not be appropriate for polar taxa (Chapman and Riddle, 2005; Holan et al., 2016; Marcus Zamora et al., 2015).

Biota in subantarctic regions, located approximately halfway between temperate regions and Antarctica, are likely to possess characteristics of each of these regions. On the one hand, like the Antarctic, the subantarctic marine environment experiences relatively cold and stable temperatures (Convey and Lebouvier, 2009). On the other hand, subantarctic tidal zonation is similar to temperate regions and unlike the Antarctic where sea ice prevents zonation of biota in intertidal and subtidal zones. Consequently, species living high on the subantarctic intertidal zone are exposed and adapted to larger fluctuations in abiotic stressors (e.g. temperature, pH and salinity). Species that are better adapted to abiotic stressors have also been found to have higher tolerance to contamination (Holan et al., 2016; Kwok and Leung, 2005). Evidence therefore suggests that species located in the subantarctic will have sensitivities to contaminants midway between the highly sensitive Antarctic species, and species of temperate regions. Despite the likelihood of many species having high sensitivity to contaminants, there is currently no ecotoxicological data published for subantarctic gastropods. This is particularly alarming, as the Southern Ocean comprises high levels of endemism (Griffiths et al., 2009).

Gastropods are highly abundant in marine ecosystems world-wide, performing important roles as grazers and as major food sources for

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higher trophic levels. Many are highly susceptible to contaminants (Grosell et al., 2007) and to stressors associated with climate change including ocean acidification (Byrne et al., 2010; Orr et al., 2005). Removal of gastropods from intertidal environments has been shown to cause top down trophic cascades by increasing algal densities and reducing space available for other invertebrate biota to colonise (Lindberg et al., 1998). For example, mass mortality of the grazing limpet *Patella vulgata,* resulting from an oil spill and its treatment with dispersant, resulted in overgrowth of brown algae, which severely impacted the remaining barnacle communities (Southward and Southward, 1978). Gastropods on subantarctic Macquarie Island constitute an important part of the intertidal community and food chain; regulating algal growth, and as prey species for many invertebrates such as isopods and sea stars, and birds such as Kelp gulls (Simpson, 1976). The trochid *Cantharidus capillaceus coruscans,* in particular, is a common food source for birds (Simpson, 1976) and crushed shells of this species can be seen in Skua (*Catharacta skua*) boluses, often observed kilometres from the intertidal zone (JH pers. obs).

Routine ecotoxicological testing with molluscs is usually conducted on bivalves or on early life history developmental stages of a range of bivalves and gastropods, with few tests done and little data therefore available on the toxicity of contaminants to adult stages of gastropods (Hunt and Anderson, 1993). The focus on early life stages reflects their often higher sensitivity to contaminants relative to adults (ANZECC/ARMCANZ, 2000; Hunt and Anderson, 1993). However, this deficiency in adult marine gastropod toxicity data needs to be addressed, due to their often high sensitivity to contaminants in comparison to many other taxa (Grosell et al., 2007; Hunt and Anderson, 1993). It is also likely that adult stages of some gastropods are challenging as toxicity test organisms due to their ability to avoid contact with test solutions. For example, the intertidal gastropod *Austrocochlea constricta* was disregarded as a test species due to its tendency to climb out of the water in treatments containing copper (Hughes et al., 2005). However, selection of suitable test species should include sensitive and ecologically important species, rather than a bias towards easily tested species (ANZECC/ARMCANZ, 2000; Hunt and Anderson, 1993). Behavioural and sublethal endpoints can be used to gain some insight into the sensitivity of species where mortality is difficult to determine (ANZECC/ARMCANZ, 2000; Hughes et al., 2005). Many studies with gastropods have adopted behavioural or sublethal endpoints such as retraction into shell (Cheung and Wong, 1999), oxygen consumption (Cheung and Wong, 1998), modifications to burying (Chapman et al., 1985) and grazing activity (Elfwing and Tedengren, 2002) as alternatives to lethal endpoints.

While generally thought of as pristine, the subantarctic has undergone several decades of human habitation and continues to be impacted by a range of contaminants. Metal contaminants, including copper, are usually associated with legacy refuse and fuel spill sites around subantarctic and Antarctic research stations (Deprez et al., 1994; Scouller et al., 2006; Stark et al., 2003). Copper is also a major pollutant in coastal areas due to its presence in sewage outfalls (Lee et al., 2010) and due to its increasing use on ship hulls since the banning of TBT (Srinivasan and Swain, 2007). Concentrations of copper may be increasing in subantarctic areas with increases in shipping activities associated with research, fishing and tourism. Additionally, copper is one of the most toxic metals to aquatic biota (Chapman and Riddle, 2005; JunFeng et al., 2014; Rosen et al., 2008). A growing body of research is aimed at determining whether polar species are particularly sensitive to copper when compared to the sensitivities of related taxa in other regions (Holan et al., 2016; King and Riddle, 2001; Marcus Zamora et al., 2015).

In this study, we determined the sensitivity of three subantarctic gastropods to copper; the littorinids, *Laevilittorina caliginosa* (Gould, 1849) and *Macquariella hamiltoni* (Smith, 1898); and the trochid, *Cantharidus capillaceus coruscans* (Hedley, 1916). This is the first study to determine the sensitivity of subantarctic gastropods to contamination. We aimed to determine appropriate test durations as well as both lethal and sublethal endpoints for each species. Behavioural endpoints are important as precursors to mortality, serving as "early warning signals" and can indicate effects of contaminants at lower concentrations. Based on previous findings with other subantarctic taxa (Holan et al., 2016), we predict that taxa living on the upper intertidal zone will be more tolerant to copper than those lower on the shore. The study location, Macquarie Island, is comparable to many other subantarctic areas with similar climates, as well as areas of the Antarctic Peninsula and southern South America. Sensitivity data from this study will therefore be applicable to the development of water quality guidelines and risk assessment procedures across the whole subantarctic region.

2. Methods

2.1. Study location and species

Gastropods used in this study were collected from subantarctic Macquarie Island (54.6167°S, 158.8500°E), which is located in the Southern Ocean, just north of the Antarctic Convergence. Sea temperatures surrounding Macquarie Island are relatively stable throughout the year, with average temperatures ranging from ~4 to 7 °C (Reynolds and Banzon, 2008). Seawater samples were taken from gastropod collection sites and verified as free from metal contamination by analysis by inductively coupled plasma optical emission spectrometry (Varian 720-ES) (ICP-OES).

The three gastropod species were collected from a range of habitats within the intertidal and subtidal zone (Fig. 1). Individuals of *Laevilittorina caliginosa* were often located in pools high on the intertidal zone, but can occur throughout the intertidal zone. Collections of this species were not specific to any one habitat. *Macquariella hamiltoni*, endemic to Macquarie Island, was collected from macroalgae in the high energy areas of the subtidal zone. *Cantharidus capillaceus coruscans* was collected from the undersides of boulders in deep tide pools as well as from crevices in the shallow subtidal. This species was also abundant on the floating fronds of *Macrocystis pyrifera* in deep water up to several hundred meters from the shore.

2.2. Toxicity tests

Toxicity tests were conducted at Macquarie Island, over the 2013/14 austral summer, and at the Australian Antarctic Division (AAD) in Tasmania, Australia, in 2015. The first round of tests with *Laevilittorina caliginosa* and *Macquariella hamiltoni* as well as both tests for *Cantharidus capillaceus coruscans* were done on Macquarie Island, while the second test for *L. caliginosa* and *M. hamiltoni* were done in Australia. Gastropods for use in tests in Australia were

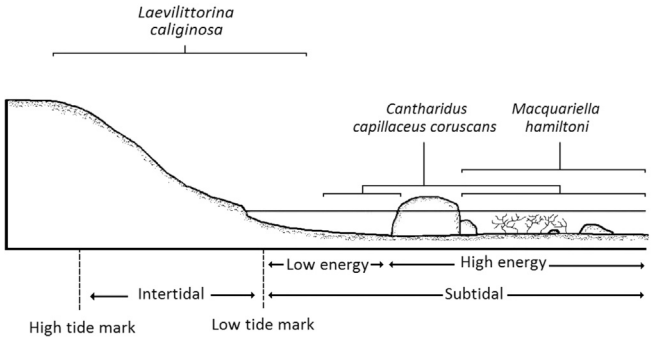

Fig. 1. Distribution on the rocky shore at subantarctic Macquarie Island of the three gastropod species used in toxicity tests in this study. Not to scale.