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Toxicity of manganese to various life stages of selected marine cnidarian species

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ABSTRACT

Manganese (Mn) pollution in marine waters is increasing and sensitivities to this metal vary widely among marine species. The aims of this study were to characterise Mn chemistry in seawater, and evaluate the toxic effects of Mn on various life stages of two scleractinian corals – the branching sp. *Acropora spathulata* and massive sp. *Platygyra daedalea*, and the anemone *Exaiptasia pallida.* Analytical and theoretical characterisation experiments showed that 97–100% of Mn (II) additions ≤ 200 mg/L in seawater were soluble over 72 h and largely assumed labile complexes. Concentrations estimated to reduce coral fertilisation success by 50% (5.5-h EC50) were 237 mg/L for *A. spathulata* and 164 mg/L for *P. daedalea*. A relatively low 72-h LC50 of 7 mg/L was calculated for *A. spathulata* larvae. In a pilot test using fragments of adult *A. spathulata*, intact coral tissue rapidly sloughed away from the underlying skeleton at very low concentrations with a 48-h EC50 of just 0.7 mg/L. For *E. pallida*, survival, tentacle retraction and reproduction were unaffected by prolonged high exposures (12-d NOEC 54 mg/L). This study provides important data supporting the derivation of separate water quality guidelines for Mn in systems with and without coral – a decision recently considered by Australian and New Zealand authorities. It demonstrates the high sensitivity of coral larvae and adult colonies to Mn and the potential risks associated with relying on other early life stage tests and/or *E. pallida* as ecotoxicological representatives of critically important scleractinian corals.

1. Introduction

1.1. Coral reef ecosystems and ecotoxicology

Increasing quantities and varieties of anthropogenic contaminants are degrading the health and resilience of coral reefs worldwide (Wooldridge and Done, 2009). Toxicity testing methods to accurately predict and assess the impacts of contaminants on coral reefs and other tropical marine ecosystems remain underdeveloped, despite the high social, economic and ecological value of these environments. In the absence of regionally-relevant toxicity data and standardised test species, the application of temperate toxicity data to tropical marine environments is accepted, albeit acknowledged as unreliable (Kwok et al., 2007; Howe et al., 2012). This surrogate approach is used in the derivation of national water quality guidelines (WQGs) by the Australian and New Zealand Environment Conservation Council (ANZECC) and the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) (2000), and international risk assessment protocols (e.g. US EPA, 2002; OECD, 2016). As human development in tropical regions continues to intensify, regulatory authorities will increasingly require access to relevant toxicity data and contaminant risk assessment and monitoring tools to ensure that coral reefs are appropriately protected (van Dam et al., 2008).

1.2. Manganese

Mn is a biologically essential trace metal, a major global commodity and an emerging marine contaminant for which ecotoxicological data are inadequate (ANZECC and ARMCANZ, 2016). Background concentrations range from 0.03 – 10 µg/L in open seawater and 0.2–26 µg/L in coastal waters, depending on local geology (CICAD, 2004). Higher concentrations (> 30 mg/L) occur under suboxic conditions (e.g. sediment porewaters, groundwater) and as a result of anthropogenic contamination from sources including runoff and leaching from metal ore extraction and processing sites, tailings dam seepage and collapse, submarine tailings disposal, channel dredging, land reclamation, urban and industrial effluents, agricultural runoff, erosion, and atmospheric deposition (CICAD, 2004).

Mn is largely used in the fabrication of steel, aluminium and other alloys, as well as various chemical applications (e.g. herbicides and

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motor fuels) and is thereby consumed by globally serving industries including construction, transport and agriculture (Hagelstein, 2009). The demand for Mn has increased dramatically during the past century with annual worldwide production rising from 500 thousand tonnes in 1900, to 17.5 million tonnes in 2015 at a rate of around 3.5% per year since 1950 (USGS, 2017). To sustain this trend, major growth in mining (and associated pollution) has occurred and is anticipated to continue primarily in tropical-subtropical regions including Asia, South America and northern Australia (USGS, 2016). Furthermore, imminent exploitation of abundant deep-sea Mn reserves throughout the Indo-Pacific is expected to result in extensive sediment resuspension and mobilisation, causing elevated dissolved Mn concentrations in the water column with unknown ecological consequences (Glover and Smith, 2003; ISA, 2017).

The neurotoxic effects of excess Mn in humans have been well documented for over a century (reviewed by ATSDR, 2012). On the contrary, evidence of Mn toxicity to marine organisms is limited. Consequently, ANZECC and ARMCANZ (2000) marine WQGs do not exist for Mn due to insufficient data to derive a reliable trigger value. As part of a recent draft revision of the guidelines, a separate "low reliability" trigger value has been recommended for Mn in systems with coral (ANZECC and ARMCANZ, 2016). This is the first time such a decision has been considered and is based on a single study by Stauber et al. (2002); cited in ANZECC and ARMCANZ, (2016) who report relatively low toxicity estimates for the common adult coral *Stylophora pistillata* (48-h EC50: 0.86 mg/L, acute NOEC: 0.51 mg/L). An unusual toxicological response was observed (for which the EC50/NOEC were calculated) referred to as "tissue sloughing", whereby the living coral tissue disconnected from the carbonate skeleton. By comparison, previous Mn toxicity estimates for marine invertebrates range from 5 mg (e.g. *Crassostrea virginica*; Calabrese et al., 1973) to 30 mg (e.g. *Mytilus edulis*; Morgan et al., 1986).

The oxidation of soluble Mn (II) to insoluble Mn (IV) forms has important ecotoxicological consequences. However, most existing studies fail to measure dissolved concentrations often reporting only total or nominal concentrations (e.g. Calabrese et al., 1973; Morgan et al., 1986; Ismail et al., 2002; Pinsino et al., 2010), which complicates interpretation of actual Mn exposures and effect levels. Knowledge of chemical speciation and bioavailability is essential when the objective is to study the fate and effects of trace metals in the environment (VanBriesen et al., 2010).

1.3. Phylum Cnidaria in ecotoxicology

Scleractinian corals are foundation species responsible for creating the physical structure that supports the outstanding biodiversity of reef ecosystems, and are highly sensitive to changes in water quality (Harrison and Booth, 2007). Nonetheless, coral ecotoxicology is limited as laboratory husbandry is typically fraught with difficulties and wild collection is inherently destructive such that consistent supplies of test organisms required for adequate replication cannot be readily obtained (Vijayavel and Richmond, 2012). Synchronous spawning among hermatypic corals maximises reproductive success and allows genetic exchange among otherwise asexual colonies (Harrison et al., 1984). It also provides large numbers of individuals for ecotoxicological testing and substantial coral toxicology work is thus dedicated to the use of early coral life stages (e.g. fertilisation success, larvae survival and development; Reichelt-Brushett and Harrison, 1999, 2005; Negri and Hoogenboom, 2011; Gissi et al., 2017; Leigh-Smith and Reichelt-Brushett, 2018). However, these methods are restricted as spawning occurs infrequently (usually annually; Harrison et al., 1984). In light of the limitations of coral ecotoxicology, the anemone *Exaiptasia pallida* (Grajales and Rodriguez, 2014) (formerly *Aiptasia pulchella*; Carlgren, 1943), which similarly nurtures intracellular relationships with *Symbiodinium* sp. yet reproduces rapidly and reliably in well-maintained aquaria, has been identified as a model cnidarian for routine ecotoxicological testing (Howe et al., 2012; Trenfield et al., 2017).

The aims of this study were to first characterise Mn solubility and speciation in seawater, then evaluate the toxic effects of Mn on early and adult life stages of two scleractinian corals – the branching species *Acropora spathulata* (Brook, 1891) and massive species *Platygyra daedalea* (Ellis and Solander, 1786), as well as the anemone *E. pallida*. Data will make important contributions to scientific understanding of the relative sensitivity of different life stages of these cnidarians, and to the development of reliable WQGs for Mn in marine systems with coral. *A. spathulata*, *P. daedalea* and *E. pallida* are sensitive organisms for which testing methods are well established (e.g. Howe et al., 2014c; Gissi et al., 2017; Leigh-Smith et al., 2018). All are commonly found throughout the tropics, whilst the distribution of *E. pallida* extends into subtropical-temperate waters; the species used in this study are therefore representative of, and data relevant to, these regions.

2. Materials & methods

2.1. Mn characterisation & speciation modelling

Mn solubility and speciation was investigated in two seawater types at a range of concentrations and time scales relevant to toxicity tests associated with this study. Analytical Mn characterisation experiments were completed using seawater collected from Heron Island, Queensland (QLD) (23° 44′ S, 151° 91′ E), and Ballina, Northern New South Wales (NSW) (28° 78′ S, 153° 59′ E), Australia. Three independent sets of six Mn concentrations (0, 5, 25, 50, 100, 150, 200 mg/L, nominal) were prepared using each seawater type and analytical grade (> 99% purity) Mn (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$; CAS: 13446-34-9). Filtered and unfiltered samples were taken for analysis (as per Section 2.4) at 24-h intervals over a 72-h aging period. In conjunction, the geochemical modelling program PHREEQC™ (v3.0) was used to predict Mn speciation in seawater at 20 mg/L and 180 mg/L, as Mn (II). Standard seawater parameters (Pilson, 1998) and Pitzer equations (suited to seawater-based models; Turner et al., 2016) were employed in the model and results were density corrected for seawater (factor $x/1.023$).

2.2. Anemone husbandry & recruitment

Healthy *E. pallida* were harvested from a naturally established population at Seaworld on the Gold Coast, QLD, and maintained prior to testing at Southern Cross University (SCU), Lismore, NSW (as per Howe, 2014). Anemones were kept in aerated glass aquaria, free of rocks and additional substrate with a 12-h:12-h photoperiod (39-W white-blue fluorescent aquarium lighting) and provided with a commercial food source (frozen *Artemia* sp.) once weekly. Water quality was maintained at constant temperature (26 ± 1 °C; using 240-W glass aquarium heaters), conductivity (54 ± 2 mS/cm), pH (8.3 ± 0.3) and dissolved oxygen (DO) (8 ± 1 mg/L) by virtue of weekly 20% exchanges completed using fresh seawater. Normal asexual reproduction occurred via pedal laceration to rear sufficient numbers of recruits of standardised sizes for toxicity testing (Cary, 1911; Howe et al., 2012).

2.3. Data quality assurance

Copper (Cu) is one of the most toxic trace metals and widely used in ecotoxicological studies as a reference toxicant. In parallel to Mn tests, corresponding Cu reference toxicity tests were completed using *E. pallida*, coral larvae and coral gametes (Table 1). Cu toxicity estimates calculated in this study were expected to fall within ranges of those provided in the literature for these test organisms to provide quality assurance of the experimental method and organism health. For further data QA/QC, Mn toxicity tests were duplicated where possible (Table 1).