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Inhibition in fertilisation of coral gametes following exposure to nickel and copper

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ABSTRACT

The mining and production of nickel in tropical regions have the potential to impact on ecologically valuable tropical marine ecosystems. Currently, few data exist to assess the risks of nickel exposure to tropical ecosystems and to derive ecologically relevant water quality guidelines. In particular, data are lacking for keystone species such as scleractinian corals, which create the complex structural reef habitats that support many other marine species. As part of a larger study developing risk assessment tools for nickel in the tropical Asia-Pacific region, we investigated the toxicity of nickel on fertilisation success in three species of scleractinian corals: *Acropora aspera*, *Acropora digitifera* and *Platygyra daedalea*. In the literature, more data are available on the effects of copper on coral fertilisation, so to allow for comparisons with past studies, the toxicity of copper to *A. aspera* and *P. daedalea* was also determined. Overall, copper was more toxic than nickel to the fertilisation success of the species tested. *Acropora aspera* was the most sensitive species to nickel (NOEC < 280 µg Ni/L), followed by *A. digitifera* with an EC10 of 2000 µg Ni/L and *P. daedalea* (EC10 > 4610 µg Ni/L). *Acropora aspera* was also the more sensitive species to copper with an EC10 of 5.8 µg Cu/L. The EC10 for *P. daedalea* was 16 µg Cu/L, similar to previous studies. This is the first time that the toxicity of nickel on fertilisation success in *Acropora* species has been reported, and thus provides valuable data that can contribute to the development of reliable water quality guidelines for nickel in tropical marine waters.

1. Introduction

The global demand and production of nickel is steadily increasing at an average rate of 5% per annum (INSG, 2016). Nickel ore occurs in two main ore types, magmatic sulphides and nickel laterites. Nickel laterites make up 60–70% of the world's nickel reserves and are formed from the extensive weathering of ultramafic rock under tropical conditions; hence, the majority of lateritic nickel ores are found between the Tropic of Cancer and the Tropic of Capricorn (Bobicki et al., 2014; Elias, 2002; Van der Ent et al., 2013).

In 2015, the Philippines, New Caledonia and Indonesia were three of the top four producers of nickel worldwide, producing 23%, 8% and 7% of global nickel, respectively (U.S. Geological Survey, 2016). Mining, smelting and transport of nickel ores within tropical Asia Pacific occurs in coastal regions of relatively small island nations, providing the potential for these activities to impact the local marine environment. Many of these nations have few environmental monitoring data and rudimentary regulatory frameworks, which limit the ability to protect tropical marine ecosystems (Mokhtar et al., 2012; Reichelt-Brushett, 2012).

The Asia-Pacific region comprises many unique and highly valuable ecosystems. As a result of the high seawater temperatures and maximum solar irradiation close to the equator, biodiversity of tropical marine species is highest within the tropical Asia Pacific (Hoeksema, 2007). In fact, the centre of coral reef biodiversity, which has been termed the Coral Triangle, includes eastern Indonesia, Malaysia, the Philippines, Timor-Leste, Papua New Guinea and the Solomon Islands (Wilkinson et al., 2016). The Coral Triangle supports 76% of the world's total coral species, with over 550 species of hard corals, of which at least 15 species are endemic to the region (Veron et al., 2009; Wilkinson et al., 2016). Coral reefs also provide valuable structural habitats and support one-third of all marine biodiversity (Harrison and Booth, 2007; Wilkinson et al., 2016).

Coral reefs throughout Southeast Asia are considered to be under

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the greatest threat of decline due to anthropogenic climate change, physical destruction, overfishing, pollution and sedimentation (Burke et al., 2012; Wilkinson et al., 2016). There have been limited field studies investigating the impact of mining activities on coral reefs and tropical marine biota. In a coral reef lagoon in New Caledonia, the concentration of nickel in suspended matter was > 7000 mg/kg, thought to have been delivered to the lagoon via natural and mining-related terrigenous inputs (Fernandez et al., 2006). While this nickel was in particulate form, and likely to be less available for accumulation by biota, the increased and long term input of this terrigenous material may increase the exchangeable fraction of metal from the sediment to the water, which may become bioavailable and be absorbed by local organisms (Fernandez et al., 2006). Dissolved nickel concentrations around the Indo-Pacific have been reported to be < 4 µg/L (Table S1) (Mokhtar et al., 2012; Srichandan et al., 2016), although at polluted sites around the globe, nickel concentrations can reach 2000 µg/L (Eisler, 1998; Pyle and Couture, 2012). Studies around New Caledonia have shown that key taxa including cephalopods (Bustamante et al., 2000), bivalves (Hedouin et al., 2009) and ascidians (Monniot et al., 1990) have elevated concentrations of nickel in their tissues in areas of increased nickel mining activity. No data are available to assess nickel accumulation in corals in response to mining. However, research within the Asia-Pacific region has shown that corals accumulate other metals in response to mining activities including zinc and lead in Papua New Guinea (Fallon et al., 2002), copper, manganese and zinc in the Philippines (David, 2003) and copper, zinc, chromium, cobalt and molybdenum in Thailand (Howard and Brown, 1987). Howard and Brown (1987) also reported nickel concentrations of 44 µg/g in the tissues of corals adjacent to a tin smelter. A study investigating trace metals in corals from the Great Barrier Reef, QLD, Australia, reported background concentrations of nickel in scleractinian corals ranging from < 0.03–0.56 µg/g (Denton and Burdon-Jones, 1986). A recent study by Hédouin et al. (2016a) used laboratory-based experiments to assess the bioaccumulation of nickel in the scleractinian coral, *Stylophora pistillata* from New Caledonia. Following a 14 day-exposure, results showed that *S. pistillata* could efficiently bioaccumulate nickel within zooxanthellae and host tissues (Hédouin et al., 2016a). While these are useful data on the accumulation of nickel in coral tissues, studies so far do not provide information on the potential toxicity of nickel to corals.

Toxicity data are required for the development of risk assessment tools such as water quality guidelines (WQGs) and bioavailability-based models. WQGs are used by governments and industry to set thresholds indicating potential risks to aquatic environments from exposure to contaminants such as metals (Wang et al., 2014). Despite evidence of increasing mining activity in the Asia-Pacific region, there has been very little advancement on the development of ecologically relevant risk assessment tools. Research into the impacts of metal contaminants on temperate marine species is extensive and consequently risk assessment tools and WQGs in these regions are well established (ANZECC/ARMCANZ, 2000; ECHA, 2008; OECD, 2011; USEPA, 2005). However, this is not the case for tropical regions, and it has been acknowledged that this lack of research in tropical marine ecotoxicology impedes development of risk assessment tools (Hudspith et al., 2017; Peters et al., 1997; Wang et al., 2014). It has also been stressed that such tools should be based on ecologically relevant data and that it may not be appropriate to apply temperate tools or guidelines to tropical settings, due to the vast differences in climate and the evolutionary distinct biota (Chapman et al., 2006; Reichelt-Brushett, 2012). Corals in particular, a key tropical taxa, are not represented in species sensitivity distributions used to derive marine WQGs.

A recent review by Gissi et al. (2016) showed that there are limited high quality data on the toxicity of nickel to key tropical marine species, including corals. Only two studies have assessed the impact of nickel on 5-h fertilisation success in two species of corals (Reichelt-Brushett and Harrison, 2005; Reichelt-Brushett and Hudspith, 2016), however, only the latter study used measured nickel concentrations. Previous studies with corals have shown that their response to metals can vary within the same species, so additional data are needed to quantify interspecies variability (Reichelt-Brushett and Harrison, 2005).

The aim of this study was to address the data gaps regarding the lack of coral-specific toxicity information (Gissi et al., 2016). We investigated the toxicity of nickel to three species of corals with widespread distribution in Indo-Pacific reefs, including the brain coral, *Platygyra daedalea* (tested previously by Reichelt-Brushett and Hudspith, 2016) of the Merulinidae family, and two species of branching coral from the Acroporidae family, *Acropora aspera* and *Acorpora digitifera*. All three species used in this study are hermaphrodite, broadcast spawning corals that release sperm and eggs into the water column for external fertilisation (Veron, 1986). Fertilisation success is an ecologically relevant endpoint to use when assessing metal toxicity, because during external fertilisation gametes are in direct contact with the water column and may be exposed to trace metals (Hudspith et al., 2017). In addition, past studies have shown that coral gametes are sensitive to metals (Hédouin and Gates, 2013; Negri and Heyward, 2001; Reichelt-Brushett and Harrison, 1999, 2005; Reichelt-Brushett and Hudspith, 2016; Victor and Richmond, 2005). The toxicity of copper to *P. daedalea* and *A. aspera* was also investigated to allow for comparison with previous studies. Although copper is an essential nutrient, it is toxic at elevated concentrations, and is found in aquatic environments due to its frequent use in many anthropogenic processes (Flemming and Trevors, 1989). Additionally, toxic effects of copper on marine organisms are frequently observed at environmentally realistic concentrations (Levy et al., 2008). Fertilisation success was measured after a 5-h exposure to nickel and copper (separately).

2. Materials and methods

2.1. General laboratory techniques and reagents

All glassware and plastic containers used in the tests were acid-washed in 10% (v/v) nitric acid (Merck) and thoroughly rinsed with demineralised water, followed by Milli-Q® water (MQ, 18.2 MΩ/cm; Merck), then soaked in natural seawater for at least 24 h.

All metal stock solutions were made volumetrically using MQ water. Copper stock solutions of 5 mg Cu/L and 100 mg Cu/L were prepared using copper (II) sulphate salt (A.R. grade, AJAX Chemicals, Australia), and acidified to 0.1% HCl (Tracepur, Merck). A nickel stock solution of 100 mg/L was made using nickel (II) chloride hexahydrate salt (A.R. grade, Chem Supply, Australia) and acidified to 0.01% HCl.

2.2. Toxicity tests with corals – 5 h fertilisation success

Toxicity test methods followed those described in Reichelt-Brushett and Harrison (1999, 2005), and Reichelt-Brushett and Hudspith (2016). Toxicity tests in this paper were conducted on Heron Island, southern Great Barrier Reef Marine Park, Australia, during a mass spawning event in November 2015. Test parameters and conditions are shown in Table 1.

Gravid coral colonies, with pigmented mature eggs (Harrison et al., 1984), were collected from the reef flat and placed in outdoor aquaria with rapid flow-through natural seawater, approximately 2–3 days before spawning was predicted to occur. Each individual colony was kept in its own tank to ensure identification and separation of egg sperm bundles from specific colonies. Three species of corals were collected and tested including the brain coral *P. daedalea* and two branching corals, *A. aspera* and *A. digitifera*.

On the afternoon prior to spawning, sperm free seawater (SFSW) (unfiltered) was collected from the reef-flat in seawater-soaked 20-L polyethylene containers. This SFSW was used to make treatment solutions in clean 500-mL polycarbonate containers by adding the required volume of metal stock to achieve the desired nominal concentration.