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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.

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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.

Keywords

Tropical marine ecotoxicology, coral reefs, Indo-Pacific, metals, risk assessment

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 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, G. 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 Hedouin 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, *P. 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; Reichelt-Brushett and Harrison, 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, 18.2MΩ/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. Sub-samples were taken from these bulk treatment solutions for analysis of total and dissolved metals (one sample per treatment). Physico-chemical parameters (including pH, salinity, dissolved oxygen and temperature) of SFSW and treatment solutions were recorded.

Spawning occurred during two nights in November 2015 with *P. daedalea* colonies spawning on the 4^{th} and *Acropora* colonies spawning on the 5^{th} (7 and 8 nights after the October full moon respectively). The nominal nickel concentration range for *P. daedalea* was 100, 500, 1000, 2500, 5000 µg Ni/L, and the range for *Acropora* species was 300, 1000, 2500, 5000, 10000 µg Ni/L. The concentration range for copper was the same for both species tested-, 10, 20, 40, 80 µg Cu/L (nominal).

Immediately after spawning, egg sperm bundles were collected from each colony and were separated by gently washing with SFSW and a 120 µm plankton mesh filter was used to separate the eggs and sperm. Test crosses were set up to ensure gametes from selected colonies were able to cross-fertilise successfully, before being used in the fertilisation experiments.

Separate experiments were performed for each metal (nickel and copper). Four to five metal treatments were set up alongside a control (SFSW), with five replicates per treatment. Bulk metal

168 treatment solutions were made at 2x the required concentration to account for dilution when added
169 to the test vials. One set of 20 mL test vials contained 4x concentrated sperm in 5mL of SFSW, to
170 account for dilution following addition of egg and treatment solution. The density of concentrated
171 spermatozoa was determined using a haemocytometer to calculate the volume required to achieve
172 a final concentration of ~ 2 x10^6 /mL in 20 mL of SFSW (following addition of egg and treatment
173 solutions). This concentration ensures ~80% fertilisation success in control treatments (Harrison and
174 Ward, 2001; Reichelt-Brushett and Harrison, 1999). In another set of 20 mL vials, ~100 eggs were
175 added into 5 mL of SFSW. Eggs were added, by glass pipette, to mini 48-well plates and
176 photographed. These photos were counted later to determine the exact number of eggs added to
177 each vial. Each treatment included five vials with sperm and five vials with eggs. Egg and sperm were
178 exposed separately by adding 5 mL of the metal solution to each vial to achieve the target nominal
179 concentration. Additional replicates were set up for physico-chemical measurements. All vials were
180 capped and allowed to incubate at room temperature (~25°C) for 30 minutes. After this time, the 10
181 mL of dosed spermatozoa solution was transferred into the 10 mL of dosed egg solution, vials were
182 capped again, placed in large zip-lock bags and placed in 20 L tubs in the outdoor aquaria with strong
183 water flow and aeration to maintain temperature and to create water movement, providing optimal
184 conditions for successful embryo development (Harrison and Ward, 2001).

185 An exposure duration of 5 h (excluding 30 minutes of separate egg/sperm exposure) was used, as
186 this has previously been found to be sufficiently long to achieve maximum fertilisation and to reach
187 early embryo stages that indicate successful fertilisation has occurred (Negri and Heyward, 2001;
188 Reichelt-Brushett and Harrison, 2005). After 5 h, vials were removed, physico-chemical parameters
189 were recorded, one replicate per treatment was selected to sub-sample for total and dissolved
190 metals, and then all vials were fixed with formalin (5 mL of treatment solution was removed from
191 each vial and replaced with 5 mL of 10% formalin). Fertilization success was observed by counting
192 the number of unfertilised eggs under a stereomicroscope. Unfertilised eggs were counted because
193 these had better structure than the fertilised eggs and enabled a more accurate assessment. The
194 number of fertilized eggs was calculated by subtracting the number of unfertilised eggs from the
195 number of eggs added at T=0. Fertilisation success was expressed as a % of control.

196 2.3. Chemical analyses

197 For all toxicity tests, 12-mL plastic syringes and 10-mL plastic vials were acid washed (10% v/v,
198 Tracepur; Merck) and rinsed with MQ in a semi clean room. All sub-samples were filtered through a
199 0.45-µm sterile filter (Sartorius Ministart® Syringe Filter, Germany) rinsed in clean seawater, into ICP
200 vials and acidified to either 0.2% (dissolved metals) or 2% (total metals) with nitric acid (Tracepur;

Merck). The vials were stored at 4°C in the dark until analysis. All samples were analysed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES, 730ES, Varian). Concentrations of metals were calculated from a matrix-matched calibration curve, using a serial dilution of an internal mixed metal standard (metals included Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, Zn). Seawater blanks and a 200 µg/L internal mixed metal drift standard were incorporated into the analysis procedure for quality assurance purposes. The mean of the dissolved measured values was taken from T=0 and T=5 h.

DOC in sub-samples of SFSW were measured after filtering through a 0.45-µm filter, collected in a glass vial and acidified with 2 mL of concentrated H_2SO_4. Analysis of DOC was done by the National Measurement Institute, Sydney, Australia. The concentration of DOC in the SFSW collected from Heron Island was 0.8 mg C/L.

2.4. Statistical analyses

Statistical analyses were undertaken using the software R (version 3.3.2, 2016-10-31) (R Core Team, 2016) in the drc package (version 3.0-1) (Ritz et al., 2015). For each species, four different models were fitted to the data, including log-logistic models with 3 and 4 parameters (LL.3, LL.4), and two Weibull models with 3 parameters (W1.3 and W2.3). The model of best fit was selected using the Akaike Information Criterion (AIC) value (model of best fit had the lowest AIC value) and by visual assessment of the curve (Table S2). The power of the drc package in R is that a suite of models can be fitted to the data at one time, allowing for comprehensive analysis of concentration-response data (Fox, 2016). The chosen model was then used to determine toxicity estimates (EC_5, EC_{10}, EC_{50}).

For comparison with past studies that have reported the no observable effect concentration (NOEC), these values were also determined in the R software. The mixtox package (v. 1.3.1) was used to run Dunnett's test (0.05 level of significance) to determine the NOEC. The GGplot2 package (v. 2.2.1) in R was used to generate a graphical representation of the data and the model used to calculate toxicity estimates. While ECx data are preferred, NOEC values are still considered for use in regulatory frameworks (Batley et al., 2014) when ECx values cannot be determined (Green, 2016).

3. Results

3.1. Quality assurance

Over the 5-h exposure, in all tests, physico-chemical parameters were within acceptable limits (Table1), measured values of: pH 8.1 ± 0.1, salinity 34 ± 0.3‰, DO 8.1 ± 0.2 mg/L (±standard error, (SE). Temperature was maintained at 25 ± 2°C in the outdoor aquaria.

Background concentrations of metals in SFSW used in all tests were below the limits of detection (LOD, Fe 0.47, Mo 0.68, Mn 0.60, Al 0.51, As 4.4, Ba 0.20, Cd 0.35, Co 0.55, Cr 0.61, Cu 0.80, Ni 2.1, Se 3.5, V 0.69 µg/L), except for Test 2 (*A. digitifera* and *A. aspera*) where Mo and Cu were detected at 0.86 µg/L and 1.2 µg/L, respectively (Table S3).

Measured dissolved nickel concentrations were within 88-97% of nominal values. The loss of nickel in test vials over 5 h was low, between 0-5% (Table S4). There was no significant difference between total and dissolved nickel, indicating that the nickel in the test solutions was in the dissolved phase (data not shown).

Measured dissolved copper concentrations were within 46-67% of nominal values. This is attributed to the loss of copper in test solutions to the glass vial walls, and potentially due to uptake by the coral gametes over 5 h (Table S4). The difference between total and dissolved copper in test solutions was between 4-23% (data not shown).

The fertilisation success for all three species met acceptability criteria (>80% fertilisation in controls, Table 1), with ≥96% fertilisation in control treatments (data not shown).

3.2. Toxicity of nickel to corals – 5 h fertilisation success

Based on the calculated endpoints and the concentration response curves (Table 2, Figures 1A-C), *A. aspera* was the most sensitive to nickel, with a NOEC of <280 µg Ni/L. For the next most sensitive species, *A.digitifera*, fertilisation success was inhibited by 10% at 2000 (1580-2420) µg Ni/L (95% confidence limits, CL). The NOEC for this species was 940 µg Ni/L (Table 2). The least sensitive species to nickel was *P. daedalea;* the NOEC was 920 µg Ni/L (Table 2). There was a very small but significant effect on fertilisation at concentrations ≥920 ug/L (Figure 1C). The slopes of the nickel concentration response curves for the three species were different; the slope for *A. digitifera* (Figure 1B) was steep, while for *A. aspera* and *P. daedalea* the slopes were more gradual (Figures 1 A and C).

3.3. Toxicity of copper to corals – 5 h fertilisation success

Copper was more toxic than nickel to coral fertilisation. Based on the EC10 values *A. aspera* was the more sensitive species, with an EC10 (95% CL) of 5.8 (1.6-10) µg Cu/L compared to *P. daedalea* at 16 (14-18) µg Cu/L (Table 2). At higher percentage effect levels, *P. daedalea* was more sensitive to copper, with an EC50 of 28 (27-30) µg Cu/L compared to *A. aspera* (EC50 of 78 (36-121) µg Cu/L) (Table 2), although this value is an extrapolation beyond the highest concentration tested (54 µg Cu/L). This is also shown in Figure 2; the slope of the concentration response curve for *P. daedalea* (Figure2B) is much steeper than that of *A. aspera* (Figure2A). In toxicity tests with *A. aspera* complete inhibition was not observed and at the highest concentration tested, fertilisation success

was >40% (Figure 2A). In contrast, the highest concentration tested for *P. daedalea* was 37 µg Cu/L resulting in fertilisation success of <30% (Figure 2B).

4. Discussion

Many corals live within a narrow range of water quality and temperature conditions and are very sensitive to changes in their environment (Harrison and Booth, 2007). Chemical communication is key in mediating processes such as reproduction and settlement which are central to the survival and persistence of coral reefs (Harrison and Wallace, 1990; Peters et al., 1997). Water quality changes may occur through anthropogenic inputs and this can disrupt key chemical interactions among reef organisms (Bielmyer et al., 2010; Peters et al., 1997). Coral gametes and larvae are particularly sensitive to changes in water quality because they are released into the environment to fertilize and undergo development where they are potentially in direct contact with anthropogenic contaminants (Reichelt-Brushett and Hudspith, 2016).

The development of ecologically relevant risk assessment tools requires toxicity data for key tropical marine species such as corals. However, there are few published toxicity data for the effect of nickel on fertilisation success in corals (Reichelt-Brushett and Harrison, 2005; Reichelt-Brushett and Hudspith, 2016). In the present study we have investigated the toxicity of nickel and copper on fertilisation success in three different species of scleractinian corals, two from the Acroporidae family (*A. aspera* and *A. digitifera*) and one from the Merulinidae family of brain corals (*P. daedalea*). *Acropora aspera* is found on upper reef slopes and lagoons, *A. digitifera* is found in shallow reef environments and *P. daedalea* is found in all areas of reef environments, commonly on back reef margins (Aeby et al. 2014a; Aeby et al., 2014b; DeVantier et al. 2014). The International Union for Conservation of Nature (IUCN) Redlist classifies *A. aspera* as a vulnerable species and *A. digitifera* as a near-threatened species (Aeby et al. 2014a; Aeby et al., 2014b). *Platygyra daedalea* is currently listed as “Least Concern” (DeVantier et al. 2014).

4.1. The toxicity of nickel to coral fertilisation success

The effect of nickel on the 5-h fertilisation success to corals varied among species, with *A. aspera* the most sensitive, followed by *A. digitifera* and *P. daedalea*. The first species tested was *P. daedalea* and the concentration range selected for this test was based on a previous study with the same species also at Heron Island (Reichelt-Brushett and Hudspith, 2016). In the present study the EC_{50} was estimated to be >4610 µg Ni/L. This differs to the results of Reichelt-Brushett and Hudspith (2016), who reported an EC_{50} of 1420 (1160-1800) µg Ni/L. Reichelt-Brushett and Harrison (2005) investigated the toxicity of nickel to the coral *G. aspera,* over two spawning events over two

296 consecutive years, at different locations. In the first experiment of that study, conducted at
297 Magnetic Island (QLD, AUS), they found that fertilisation success of *G. aspera* gametes was >83% in
298 all treatments, including the highest nickel treatment of 2000 µg Ni/L. The following year,
299 experiments were completed at One Tree Island (QLD, AUS) and a significantly lower fertilisation
300 rate occurred in nickel treatments ≥ 100 µg Ni/L. At the highest concentration tested (2000 µg Ni/L),
301 fertilisation success was 60%, however these estimates were based on nominal nickel
302 concentrations only. That study demonstrated that the response of coral fertilisation to metal
303 exposure can vary within the same species between locations. In addition, pre-exposure may be an
304 important factor to consider with respect to location of coral colonies that are selected for toxicity
305 testing. Reichelt-Brushett and Harrison (2005) tested corals from Magnetic Island, which is close to
306 Townsville Harbour, 8 km from the coast and close to anthropogenic contaminant sources and found
307 the corals were less sensitive than corals from One Tree Island which is located in the relatively
308 pristine Southern Great Barrier Reef Marine Park, 96 km offshore from the coast.

309 In the present study, on the second night of spawning a higher concentration range of nickel was
310 selected to ensure the full response would be captured. *Acropora aspera* was the most sensitive
311 species with a NOEC value of <280 µg Ni/L, however, EC_5 and EC_{10} values could not be calculated
312 because there were not two or more partial responses between the control and the NOEC. *Acropora*
313 *digitifera* was the next most sensitive species to nickel, with a NOEC value of 940 µg Ni/L and an EC_{10}
314 of 2000 µg Ni/L (Table 2). This is the first report on the toxicity of nickel to any *Acropora* species.
315 Given that the genus *Acropora* is the most widespread and contains the largest number of species of
316 reef-building corals (Wallace, 1999), including data for *Acropora* species increases the confidence of
317 nickel ecotoxicity thresholds that are applied to tropical marine ecosystems.

318 From the data reported in this study and two other studies investigating the effect of nickel on coral
319 fertilisation (Table 3), it is evident that sensitivity of coral gametes to nickel is variable between
320 species, but also variation exists within populations of one species. There is only one other study
321 that has investigated the toxicity of nickel to early life stages of coral. Goh (1991) studied the effect
322 of nickel on larval survival and settlement in the coral *Pocillopora damicornis*, measuring effects
323 during a recovery period following exposure to nickel for 12-96 h. The LC_{50} after a 40-h recovery
324 period was >9000 µg Ni/L. Settlement was a more sensitive endpoint, with significantly reduced
325 settlement rates after 9 days recovery from exposure to 1000 µg Ni/L (Goh, 1991). It is difficult to
326 make direct comparisons with the results obtained in the present study with that of Goh (1991)
327 because *P. damicornis* employs different reproductive strategies than corals of the genus *Acropora*
328 or *Platygyra* which are broadcast spawners that release eggs and sperm into the water column
329 (Harrison and Wallace, 1990). *Pocillopora damicornis* is a brooder; it broods sexually or asexually

generated larvae which are then released into the sea (Ayre et al., 1997). Additionally, different types of exposures, endpoints and the age of the test organism were used among the studies. In general it appears that early life stages of corals may be relatively insensitive to nickel, with most effects observed above 1000 µg Ni/L.

There are few studies that report the concentrations of nickel in seawater around the Indo-Pacific region (Table S1), though based on limited information, concentrations of nickel in seawater are typically < 4 µg/L (Mokhtar et al., 2012; Srichandan et al., 2016) and globally in contaminated coastal areas, concentrations of nickel have been reported between 50-2000 µg/L (Eisler, 1998; Pyle and Couture, 2012). Elevated concentrations of nickel of up to 7000 mg/kg have been detected in marine sediments around New Caledonia (Fernandez et al., 2006; Hedouin et al., 2009). In our study, toxic effects on coral fertilisation occurred at concentrations well above environmentally relevant concentrations for nickel in seawater around the Indo-Pacific (Table S1). However, at very polluted sites, it is possible that fertilisation of coral gametes could be impaired at high nickel concentrations.

4.2. Toxicity of copper to coral fertilisation success

Copper is more toxic to coral fertilisation than nickel. The response to copper exposure is variable between species, with effects on fertilisation success between 11-261 µg Cu/L (based on EC_{50} values, Table 3). The least sensitive species to copper is a soft coral, *Lobophytum compactum* (EC_{50} 261 µg Cu/L) (Reichelt-Brushett and Michalek-Wagner, 2005); this is the only toxicity data available for a soft coral. The EC_{50} values for hard corals range from 11-145 µg Cu/L (Table 3).

The results in this study for *P. daedalea* (EC_{50} 28 µg Cu/L) are similar to that of Reichelt-Brushett and Hudspith (2016) who estimated an EC_{50} of 33 µg Cu/L. However the EC_{10} values differ; 16 µg Cu/L in this study, compared to 1.4 µg Cu/L estimated by Reichelt-Brushett and Hudspith (2016). In a more recent study by Hudspith et al. (2017) the response of *P. daedalea* to copper was different again, with the EC_{50} estimated as 73 µg Cu/L despite use of the same methods and study location (Heron Island) as that used by Reichelt-Brushett and Hudspith (2016).

Several other studies have assessed copper toxicity on fertilisation success in brain corals (Table 3). For corals of the genus *Goniastrea,* toxicity estimates for 50% inhibition in fertilisation range from 14.5-25 µg Cu/L (Reichelt-Brushett and Harrison, 1999; Reichelt-Brushett and Harrison, 2005). Heyward (1988) reported NOEC values of ~ 10 µg Cu/L for the corals *P. ryukyuensis* and *Favites chinensis*. NOEC values in other studies range from 2 – 13 µg Cu/L, and our result (NOEC value for *P. daedalea* of 9 µg Cu/L) was in good agreement with these studies. Conversely, Kwok et al. (2016) found the coral *P. acuta* to be much less sensitive to copper with fertilisation success inhibited by

362 50% (EC_{50}) between 92 - 145 µg Cu/L (based on nominal concentrations). This response may be due
363 to the coral colonies being pre-exposed to elevated concentrations of copper in seawater around
364 Hong Kong (Kwok et al., 2016).

365 There are more data available on the effect of copper on fertilisation success in corals from the
366 Acroporidae family (Table 3). Only one study for the coral *M. capitata* provided EC_{10} estimates of 9 -
367 15 µg Cu/L over three consecutive tests (Hédouin and Gates, 2013). This is similar with our study
368 where fertilisation success in *A. aspera* was inhibited by 10% at 5.8 (1.6-10) µg Cu/L. The EC_{50}
369 estimates for Acroporidae corals range from 15 – 75 µg Cu/L (Hédouin and Gates, 2013; Negri and
370 Heyward, 2001; Puisay et al., 2015; Reichelt-Brushett and Harrison, 2005; Victor and Richmond,
371 2005) (Table 3). The results from our study for *A. aspera* (EC_{50} 78 µg Cu/L) were similar to those of
372 previous studies, and are most similar to results obtained by Puisay et al. (2015) for *A. cytherea* and
373 *A. pulchra* with EC_{50} values of 69.4 and 75.4 µg Cu/L, respectively. Caution must be taken when
374 making comparisons with other studies, particularly where the test methods vary (Hudspith et al.,
375 2017). This is the case with the study by Puisay et al. (2015) and Victor and Richmond (2005) who
376 exposed gametes to contaminants simultaneously, unlike in our study and that of Reichelt-Brushett
377 and Harrison (1999, 2005), Hedouin and Gates (2013), Reichelt-Brushett and Hudspith (2016) and
378 Hudspith et al., (2017), among others, where the test methods exposed egg and sperm separately
379 prior to combining and monitoring fertilisation success after further exposure for 3-5 h.

380 4.3. Variability in sensitivity to metals between different coral endpoints

381 Our results and other published data show that there is considerable variability in the sensitivity of
382 corals to metals (Table 3). It is difficult to distinguish patterns among different families or groups of
383 corals due to the lack of sufficient data and the use of different test methods. However, in general it
384 appears that Acroporidae corals are more sensitive to nickel than brain corals, and the reverse is
385 found for copper. The sensitivity of a species to a metal contaminant is both species and metal
386 specific. The differences in toxicity of metals to coral gametes could be due to different gamete
387 ultrastructure and morphology, biochemical processes and underlying tolerances to stressors
388 between species (Harrison, 1990; Hudspith et al., 2017). To understand these differences in
389 sensitivities, further research into the mechanisms of metal toxicity on external invertebrate
390 fertilisation is required (Hudspith et al., 2017).

391 Some previous studies have assessed the effect of copper on coral embryo viability, larval survival,
392 motility or swimming activity and metamorphosis or settlement (Table 3). In this study we also
393 attempted to assess the effect of nickel and copper on embryo viability by continuing exposure of
394 gametes from fertilisation up to 10 h, and also larval survival from fertilisation up to 72 h. This was

only tested with *P. daedalea* for nickel. In the 10-h exposure no significant response was observed (data not shown). There is only one other published study that has also continued exposure of embryos to copper following fertilisation. Victor and Richmond (2005) exposed the gametes of *A. surculosa* to copper and found that between 5 and 12 h, the EC_{50} decreased nearly four times from 45 µg Cu/L to 11 µg Cu/L. In the present study, in experiments where larval survival was monitored from fertilisation up to 72 h, no concentration response relationship was observed, although survival in controls was >65% (Table S5, Figure S1). Investigating this endpoint further would require method development, particularly around the issue of renewing treatment solutions to maintain optimal water quality conditions. Based on the literature, larval survival does not appear to be more sensitive to copper than fertilisation success, with EC_{50} values ranging from 80-198 µg Cu/L. Settlement may be a slightly more sensitive endpoint with 50% effects observed in *Acropora* species between 26-110 µg Cu/L (Negri and Heyward, 2001; Negri and Hoogenboom, 2011; Reichelt-Brushett and Harrison, 2000). The motility of coral larvae appears to be a more sensitive endpoint with EC_{50} values ranging from 22-48 µg Cu/L (Kwok et al., 2016; Reichelt-Brushett and Harrison, 2004), suggesting that further investigation of this endpoint's sensitivity and reproducibility is desirable.

4.4. Toxicity testing with coral gametes

Our study and the recent studies of Reichelt-Brushett and Hudspith (2016) and Hudspith et al., (2017) demonstrated that the same species of coral from the same location may show variations in sensitivities to metals. It is unlikely that these differences are due to experimental approach as the standard coral fertilisation methods, originally based on Reichelt-Brushett and Harrison (1999, 2005) were employed in all three studies. In corals, the gametogenesis cycle occurs over approximately five to nine months (Harrison and Wallace, 1990). Changes in environmental conditions due to natural or anthropogenic stressors (e.g. increase water temperature, coral bleaching, pollution etc) can influence the reproductive cycles and viability of coral gametes (Harrison and Ward, 2001; Hudspith et al., 2017; Ward et al., 2002). Past studies have demonstrated that the plasticity of coral gametes (e.g. egg size, no. of eggs per bundle) is linked to the environmental conditions in which the parent colonies inhabit, and the month of spawning (Hédouin and Gates, 2013; Padilla-Gamiño and Gates, 2012; Padilla-Gamiño et al., 2011). Phenotypic variability in coral gametes may enable coral species to cope with and adjust to changes in environmental conditions and stressors (Hédouin and Gates, 2013). If there is inherent variability in coral gametes, then their susceptibility to metal exposure is likely to vary temporally and between individuals within the same species (Hudspith, Reichelt-Brushett, & Harrison, 2017). Future studies should determine the variability in sensitivity of

individual colonies over time. This would require tagging colonies and testing the gametes from the same colonies over multiple years, or spawning events.

An additional factor that could influence the variability of gamete sensitivity to metals is sperm concentration. Fertilisation success is determined by sperm concentration; with both too little and too many sperm resulting in decreased fertilisation (Marshall, 2006). Field and laboratory studies have shown that in several species of scleractinian corals, optimal fertilisation success (i.e. ~ 100% fertilisation) occurs between 10^5 - 10^6 sperm/mL (Oliver and Babcock, 1992; Willis et al. 1997). In this study and many previous studies investigating toxicity of metals to coral fertilisation, the sperm concentration (2 x10^6 sperm/mL) used in toxicity tests exceeds this concentration. High concentrations of sperm can result in polyspermy that can reduce fertilisation success. Exposure to metals could therefore potentially reduce sperm numbers, thereby increasing fertilisation because polyspermy is reduced (Marshall, 2006). Metal toxicants can affect different aspects of fertilisation including sperm survival, inhibition of the proportion of egg to sperm contact or by impeding polyspermy blocks in eggs (Hudspith et al., 2017; Marshall, 2006). To investigate the effects of sperm concentration on sensitivity to toxicants it has been proposed that ecotoxicological studies on coral fertilisation should utilise a range of ecologically relevant sperm concentrations (Jones et al., 2015; Marshall, 2006). The aim of the present study was to compare our results with previous studies and so the same methods, including sperm concentration, were used. It has been shown that at this sperm concentration (2 x10^6 sperm/mL) fertilisation success in controls (unexposed gametes) is ≥80% suggesting optimal conditions (Hudspith et al., 2017; Reichelt-Brushett and Hudspith, 2016; Reichelt-Brushett and Harrison, 2005, 1999).

The available coral fertilisation toxicity data are difficult to compare directly across multiple studies due to the differences in methodologies used. The sensitivity of a species to a metal contaminant can also be influenced by the experimental design. The majority of studies on the toxicity of metals to coral fertilisation use standardized methods originally described in Reichelt-Brushett and Harrison (1999, 2005), and our study followed the same protocol in order to verify and compare our results with previous studies. A recent review by Hudspith et al. (2017) has highlighted the need to standardize toxicity testing methods for fertilisation assays with broadcast spawning invertebrates. Key issues that need to be considered to harmonise methods include exposing eggs and sperm together or separately, the egg-to-sperm ratio, the egg/sperm densities, the type of diluent/control water used in the test and the duration of exposure (Hudspith et al. 2017).

5. Conclusions

We have shown that the sensitivity of coral gametes to metals is both species and metal specific. Based on the data derived in this study and previous publications, copper is more toxic to coral fertilisation than nickel; however the dataset for nickel is limited. In general the data reported in this study are in good agreement with previous studies, however the sensitivity of coral gametes to metals varies between individuals and spatially. This may be part of the natural variation and plasticity of coral gametes development which may vary from year-to-year depending on the conditions of the adult colony. Further research is required to understand the natural variability in the sensitivity of coral gametes to metals between colonies of the same species, between species, and differences over time and geographical location.

Future research should also investigate the toxicity of nickel to different life stages including coral larvae and adult colonies and assess how *in situ* coral populations subjected to gradients of contamination respond to metal exposure. It would be valuable to increase our understanding of how nickel impacts the early stages of larvae settlement and metamorphosis and also how nickel interacts with the coral animal and its associated microbiota, including the mutualistic *Symbiodinium*.

The methodologies applied in this study meet the criteria established by government, industry and regulators (e.g. use of ecologically relevant endpoints and measured metal concentrations) for the use of high quality data in water quality guideline development. This study has provided the first data on the toxicity of nickel to fertilisation success in corals of the genus *Acropora*. This, together with the additional data for *P. daedalea,* can contribute to the inclusion of corals in the development of ecologically relevant water quality guidelines for nickel in tropical marine waters.

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Table 1. Toxicity test conditions for 5-h fertilisation tests with corals

Toxicity test parameters	
Temperature	25 ± 2°C
pH	8.1 ± 0.1
Salinity	34 ± 1‰
Conductivity	51 ± 1 mS/cm
Dissolved oxygen	>8 mg/L
Light	Ambient natural light
Test type	Static no renewal
Test duration	5 h (+ 30 min of separate egg and sperm exposure)
Test chamber	20 mL glass scintillation vials
Test solution volume	20 mL
Age of test organism	Gametes
Initial Spermatozoa density	2 x10^6/mL
Initial No. eggs	~100
No. replicate chambers per treatment	5
Control/diluent water	Natural, sperm free seawater
Test endpoint	Fertilisation success
Test acceptability	>80% fertilisation in controls

Table 2. Toxicity of nickel and copper to fertilisation success in corals following a 5-h exposure. Toxicity estimates and the no observable effect concentration (NOEC) calculated using measured dissolved (0.45 µm filtered) metal presented in µg/L. Values in parentheses are 95% confidence limits. Estimates calculated using the drc package in R.

	Nickel				Copper			
Species	EC_5	EC_{10}	EC_{50}	NOEC	EC_5	EC_{10}	EC_{50}	NOEC
Acropora aspera[a,b]	NC	NC	>9220	<280	3.3 (0.3-6.2)	5.8 (1.6-10)	78 (36-121)	<6
Acropora digitifera[b]	1680 (1260-2110)	2000 (1580-2420)	4350 (3830-4870)	940	NT	NT	NT	NT
Platygyra daedalea[a]	>4610	>4610	>4610	920	13 (10-15)	16 (14-18)	28 (27-30)	9

[a] Weibull model 1.3 used to calculate toxicity estimates. Weibull 1.3 used to calculate toxicity estimates for nickel and *A. aspera*
[b] Weibull model 2.3 used to calculate toxicity estimates. Weibull 2.3 used to calculate toxicity estimates for copper and *A. aspera*
NC, not calculated
NT, not tested

Table 3. The effect of nickel and copper on corals. Table modified from Hudspith et al., (2017). Toxicity estimates and NOEC values presented as metal concentration in µg/L. Values in parentheses are 95% confidence limits or ± standard error.

Species	Endpoint	Metal Salt	Diluent[a]	EC_{10}	EC_{50}	NOEC	Exposure characteristics[b]	Study
Nickel								
Acroporidae								
Acropora aspera	Fertilisation success	$NiCl_2.6H_2O$	SFSW	NC	>9220	<280	(Gametes 30 min) +5 h	This study
A. digitifera				2000 (1580-2420)	4350 (3830-4870)	940		
Merulinidae								
Platygyra daedalea	Fertilisation success	$NiCl_2.6H_2O$	SFSW	>4610	>4610	920	(Gametes 30 min) +5 h	This study
P. daedalea	Fertilisation success	$NiCl_2$	SFSW	NR	1420 (1160-1800)	NR	(Gametes 30 min) +5 h	Reichelt-Brushett and Hudspith (2016)
Goniastrea aspera	Fertilisation success	$NiCl_2$	SFSW	NR	>2000	NR	(Gametes 30 min) +5 h	Reichelt-Brushett and Harrison (2005)
Pocilloporidae								
Pocillopora damicornis	Larval settlement and survival	$NiCl_2.6H_2O$	F	NR	9000[c]	NR	Planulae larvae 12, 24, 48, 96 h	Goh (1991)
Copper								
Acroporidae								
A. aspera	Fertilisation success	$Cu(SO_4)_2$	SFSW	5.8 (1.6-10)	78 (36-121)	<6	(Gametes 30 min) +5 h	This study
A. cytherea	Fertilisation success	$Cu(HNO_3)_2$	F	NR	69.4	NR	Gametes 4.5 h	Puisay et al. (2015)
A. longicyathus	Fertilisation success	$CuCl_2$	SFSW	NR	15.2 (12-19.2)	15.3	(Gametes 30 min) +5 h	Reichelt-Brushett and Harrison (2005)
A. millepora	Fertilisation success	$CuCl_2$	F	NR	17.4 (± 1.1)[d]	NR	Gametes 4 h	Negri and Heyward (2001)
	Larval metamorphosis (settlement)			NR	110 (± 20)	NR	7 d old larvae 24h	

Species	Endpoint	Metal Salt	Diluent[a]	EC_{10}	EC_{50}	NOEC	Exposure characteristics[b]	Study
	Larval metamorphosis (settlement)	$CuCl_2$	F	NR	26 (±0.98)	NR	7 d old larvae, 6 h pre-exposure, + 18 h with CCA[e]	Negri and Hoogenboom 2011
A. pulchra	Fertilisation success			NR	75.4	NR	Gametes 4.5 h	Puisay et al. (2015)
A. surculosa	Fertilisation success	$CuSO_4$	F	NR	45.2	NR	Gametes 5 h	Victor and Richmond (2005)
				NR	11.4	NR	Gametes 12 h	
A. tenuis	Fertilisation success	$CuCl_2$	SFSW	NR	39.7 (36-43.7)	33.5	(Gametes 30 min) +5 h	Reichelt-Brushett and Harrison (2005)
	Larval metamorphosis (settlement)	$CuCl_2$	F	NR	32 (±0.86)	NR	7 d old larvae, 6 h pre-exposure, + 18 h with CCA[e]	Negri and Hoogenboom (2011)
	Larval metamorphosis (settlement)	$CuCl_2$	SFSW	NR	35 (32-37)	20 (nominal)	5 d old larvae, 48 h	Reichelt-Brushett and Harrison (2000)
A. tumida	Larval survival	$Cu(SO_4)_2$	ASW	5.8 (0.1-690)[d]	80 (11-590)[d]	NR	Planula larvae, 24 h	Bao et al. (2011)
Montipora capitata	Fertilisation success	$Cu(HNO_3)_2$	F	9-15.1	16.6-31.7	NR	Gametes 3 h	Hedouin and Gates (2013)
M. verrucosa	Adult coral survival	NR	NR	NR	48	NR	NR	Howard et al. (1986), from Negri and Heyward (2001)
Merulinidae								
G.aspera	Fertilisation success	$CuCl_2$	SFSW	NR	14.5	2	(Gametes 30 min) +5 h	Reichelt-Brushett and Harrison (1999)
				NR	18.5 (12-19.2)	12.8		Reichelt-Brushett and Harrison (2005)
	Larvae survival	$CuCl_2$	SFSW	NR	34 (19-62)	NR	5 d old larvae, 72 h	Reichelt-Brushett and Harrison (2004)
				NR	82 (54-123)	NR	6 d old larvae, 72 h	
	Larvae motility			NR	22 (21-23)	NR	4-6 d old larvae, 48 h	
G. retiformis	Fertilisation success	$CuCl_2$	SFSW	NR	24.7 (15.5-30)	10	(Gametes 30 min) +5 h	Reichelt-Brushett and Harrison (2005)

Species	Endpoint	Metal Salt	Diluent[a]	EC_{10}	EC_{50}	NOEC	Exposure characteristics[b]	Study
P. daedalea	Larvae motility	$CuCl_2$	SFSW	NR	36 (33-39)	NR	4-6 d old larvae, 24 h	Reichelt-Brushett and Harrison (2004)
	Fertilisation success	$Cu(SO_4)_2$	SFSW	16 (14-18)	28 (27-30)	9	(Gametes 30 min) +5 h	This study
		$NiCl_2$		NR	73	NR		Hudspith and Reichelt-Brushett (2017)
				1.4	33 (30-37)	NR		Reichelt-Brushett and Hudspith (2016)
P. acuta	Fertilisation success	$CuCl_2$	F	NR	92.1-145	NR	(Gametes 30 min) +5 h	Kwok et al. (2016)[f]
	Larval survival			NR	102-110	NR	4 d old larvae, 48 h	
				NR	101-107	NR	4 d old larvae, 96 h	
	Larvae metamorphosis (settlement)			NR	NC, no effects observed up to 200 µg Cu/L	NR	7 d old larvae, 48 h exposure, 48 h clean seawater for settlement	
	Swimming activity			NR	45.4-47.7	NR		
	Settled larvae growth		ASW	NR	NC, no effects observed up to 200 µg Cu/L	NR	Newly settled recruits (3 w old), 8 weeks	
P. ryukyuensis *Favites chinensis*	Fertilisation success	NR	NR	NR	<100	~10	NR	Heyward (1988) from Negri and Heyward (2001)
Pocilloporidae								
P. damicornis	Larval survival at 27°C	$CuCl_2$	F	NR	198	NR	96 h exposure	Hedouin et al. (2016)b
	at 30°C			NR	141	NR		
	Adult survival at 24°C			NR	251	NR	Nubbins, 2-4 cm length	

Species	Endpoint	Metal Salt	Diluent[a]	EC_{10}	EC_{50}	NOEC	Exposure characteristics[b]	Study
	at 27°C			NR	175	NR	96 h exposure	
	Larval survival	NR	NR	NR	87	NR	48 h	Esquivel (1986), from Kwok et al. (2016)
					57		96 h	
Alcyoniidae								
Lobophytum compactum	Fertilisation success	$CuCl_2$	SFSW	NR	261 (208-328)	69	(Gametes 30 min) +10 h	Reichelt-Brushett and Michalek-Wagner (2005)

NR, not reported
NC, not calculated
[a] Diluent: SFSW = sperm free natural unfiltered seawater, F = filtered natural seawater, ASW = Artificial seawater
[b] Exposure Characteristics: (Gametes 30 min) + 5 h = gametes exposed separately for 30 min then combined for 5 h
Gametes 3, 4.5, 5 h = gametes exposed simultaneously for set time
[c] Toxicity estimate calculated during recovery period
[d] Nominal metal concentrations used
[e] CCA = Crustose coralline algae
[f] Tests conducted between 26-30°C

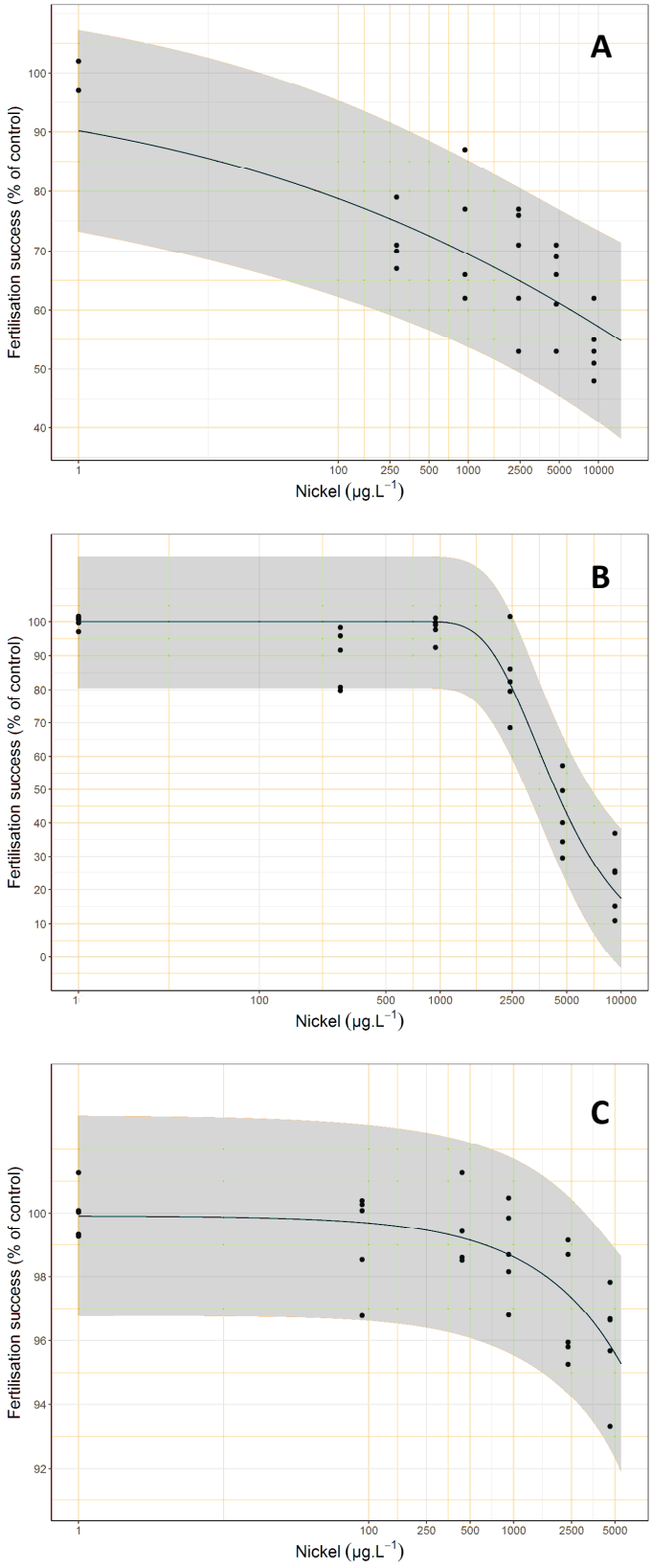


Figure 1. Toxicity of nickel to fertilisation success (as a % of control) in the corals A) *Acropora aspera*, B) *Acropora digitifera* and C) *Platygyra daedalea*. The grey ribbon shows the 95% confidence limits calculated from the model (black line). Each point represents one replicate. Data are from one individual toxicity test.

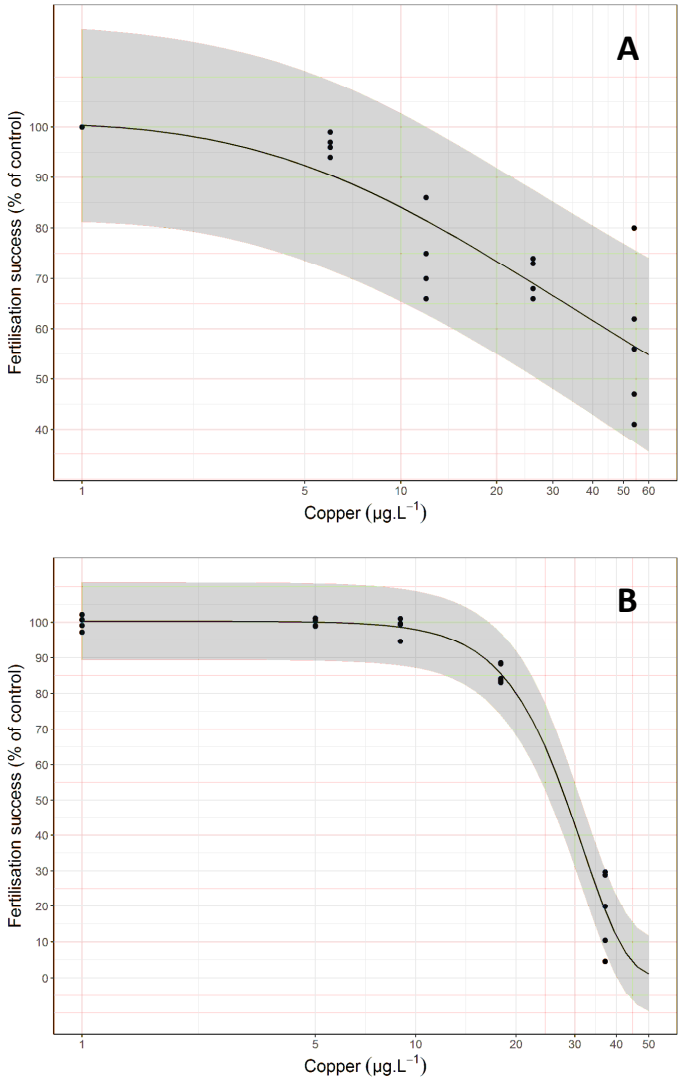


Figure 2. Toxicity of copper to fertilisation success (as a % of control) in the corals A) *Acropora aspera*, B) *Platygyra daedalea*. The grey ribbon shows the 95% confidence limits calculated from the model (black line). Each point represents one replicate. Data are from one individual toxicity test.

Supplementary material

Table S1. Environmental concentrations of nickel around tropical coral reef locations in Asia Pacific

Location	Sample	Ni	Study
Compiled from Thailand, Australia, Philippines	Sediment	74-123 µg/g	(Peters, Gassman, Firman, Richmond, & Power, 1997)
Sabah, Malaysia	Seawater	0.4-1.9 µg/L	(Mokhtar, Praveena, Aris, Yong, & Lim, 2012)
	Sediment	20-60 µg/g	
Manila Bay, Philippines	Sediment	10-19 µg/g dw	(Prudente, Ichihashi, & Tatsukawa, 1994)
Bay of Bengal, India	Seawater	0.021-3.56 µg/L	(Srichandan et al., 2016)
New Caledonia	Sediment	5-900 µg/g dw	(Hédouin et al., 2009)
Noumea, New Caledonia	Sediment	28-1879 µg/g	(Monniot, Monniot, & Laboute, 1991)
Boulari Bay and Saint Marie Bay, Noumea, New Caledonia	Sediment	7000 mg/kg	(Fernandez et al., 2006)
Townsville, Australia	Sediment	5.5-285 nM/g dw	(Esslemont, 2000)

dw = dry weight

Table S2. A list of the models and the corresponding Akaike Information Criterion (AIC) values used in the drc package in R. Four different models were fitted to each data set (species and metal). The model of best fit was chosen based on the lowest AIC value, and by visual assessment of the curve. The selected model was then used to determine toxicity estimates for each species and metal tested.

Species	Acropora aspera		Acropora digitifera		Platygyra daedalea	
Metal	Nickel	Copper	Nickel	Copper	Nickel	Copper
Model	AIC values					
Weibull 1.3	210	185	232	NT	111	156
Weibull 2.3	211	183	223		268	157
Log Logistic 3	211	185	227		112	157
Log Logistic 4	212	185	223		112	159

NT = not tested

Table S3. Background concentrations (μg/L) of metals in seawater used in toxicity tests. LOD – limit of detection reported in μg/L.

Metal	Fe	Mo	Mn	Al	As	Ba	Cd	Co	Cr	Cu	Ni	Se	V
LOD	*0.47*	*0.68*	*0.60*	*0.51*	*4.4*	*0.20*	*0.35*	*0.55*	*0.61*	*0.80*	*2.1*	*3.5*	*0.69*
Test													
1	<0.47	<0.68	<0.60	<0.51	<4.4	<0.2	<0.35	<0.55	<0.61	<0.80	<2.1	<3.5	<0.69
2	<0.47	**0.86**	<0.60	<0.51	<4.4	<0.2	<0.35	<0.55	<0.61	**1.2**	<2.1	<3.5	<0.69

Figures in bold indicate values above the limit of detection

Table S4. Nominal and measured metal concentrations used in 5-h fertilisation toxicity tests with scleractinian corals. All values are reported in µg/L, except where otherwise stated. Note, the same concentration range was tested for *A. aspera* and *A. digitifera* so only one set of metal subsamples was analysed.

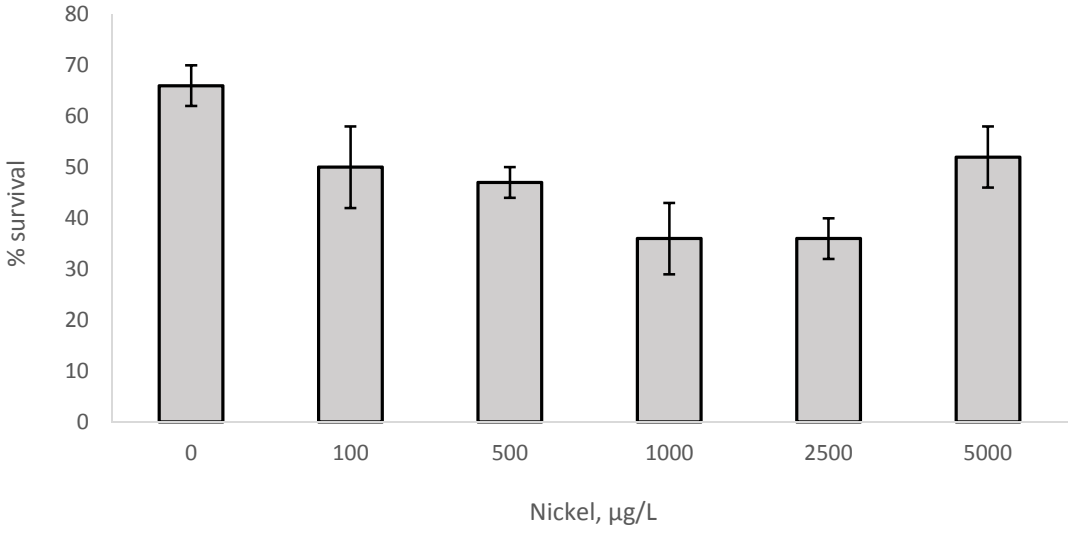
Species	Nominal	Dissolved measured T=0 h	Dissolved measured T=5 h	Metal loss over 5 h (%)
		Nickel		
Acropora aspera and *Acropora digitifera*	300	285	271	4.9
	1000	950	938	1.2
	2500	2431	2424	0.3
	5000	4786	4683	2.2
	10000	9356	9090	2.8
Platygyra daedalea	100	93	91	2.2
	500	445	433	2.6
	1000	932	907	2.7
	2500	2348	2369	-0.9
	5000	4632	4595	0.8
		Copper		
Acropora aspera and *Acropora digitifera*	10	7.8	4.0	48
	20	17	7.8	55
	40	34	19	45
	80	71	37	48
Platygyra daedalea	10	6.4	3.7	42
	20	11	7.9	26
	40	15	22	-49
	80	39	35	9.0

Larval survival from fertilisation

Table S5. Toxicity test conditions for larval survival experiment with *Platygyra daedalea*

Toxicity test parameters	
Temperature	25 ± 2°C
pH	8.1 ± 0.1
Salinity	34 ± 1‰
Conductivity	51 ± 1 mS/cm
Dissolved oxygen	>8 mg/L
Light	Ambient natural light
Test type	Static, renewal at 10 h and 36 h (after fertilisation)
Test duration	72 h
Test chamber	20 mL glass scintillation vials
Test solution volume	20 mL
Age of test organism	Gametes
Initial Spermatozoa density	2 x10^6/mL
Initial No. eggs	~100
No. replicate chambers per treatment	5
Control/diluent water	Natural, sperm free seawater
Test endpoint	Larval survival

Figure S1. The effect of Ni on larval survival of *Platygyra daedalea* following 72-h exposure from fertilisation



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