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Adult Corals Are Uniquely More Sensitive to Manganese Than Coral Early-Life Stages

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Abstract: Manganese (Mn) is an essential element and is generally considered to be one of the least toxic metals to aquatic organisms, with chronic effects rarely seen at concentrations below 1000 µg/L. Anthropogenic activities lead to elevated concentrations of Mn in tropical marine waters. Limited data suggest that Mn is more acutely toxic to adults than to early life stages of scleractinian corals in static renewal tests. However, to enable the inclusion of sufficient sensitive coral data in species sensitivity distributions to derive water quality guideline values for Mn, we determined the acute toxicity of Mn to the adult scleractinian coral, *Acropora muricata*, in flow-through exposures. The 48-h median effective concentration was 824 µg Mn/L (based on time-weighted average, measured, dissolved Mn). The endpoint was tissue sloughing, a lethal process by which coral tissue detaches from the coral skeleton. Tissue sloughing was unrelated to superoxidase dismutase activity in coral tissue, and occurred in the absence of bleaching, that is, toxic effects were observed for the coral host, but not for algal symbionts. We confirm that adult scleractinian corals are uniquely sensitive to Mn in acute exposures at concentrations 10–340 times lower than those reported to cause acute or chronic toxicity to coral early life stages, challenging the traditional notion that early life stages are more sensitive than mature organisms. *Environ Toxicol Chem* 2023;42:1359–1370. © 2023 Commonwealth Scientific and Industrial Research Organisation. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Manganese (Mn) is a biologically essential metal and the tenth most abundant element in the Earth's crust (Howe et al., 2004). The average crustal composition is 0.1%, which varies according to local geology (e.g., basanite: 1400 mg/kg; shale: 600 mg/kg; limestone: 550 mg/kg; Gilkes & McKenzie, 1988). Recent high-quality data from the GEOTRACES programme found that background dissolved Mn concentrations in intermediate to deep ocean ranged from 0.005 to 0.008 µg/L, and that surface concentrations were higher, reaching up to 2 µg/L (dissolved) in some instances (Mawji et al., 2015; Schlitzer, 2021; van Hulten et al., 2016). Total concentrations in natural surface waters, including rivers that discharge into the ocean, rarely exceed 1000 µg/L (Howe et al., 2004), but have ranged from <1 to 1700 µg/L (Reimer, 1999). Higher dissolved concentrations of Mn (>30 mg/L) occur under suboxic conditions (e.g., in sediment porewaters and groundwater), and upward migration of the dissolved Mn can lead to production of Mn-rich particles in the slightly oxygenated overlying water (see Trefry et al., 1984).

Manganese is used in the production of steel, aluminum, and other alloys, in glass production, and in agrofertilizers (Pinsino et al., 2012). The total global production volume of Mn has increased from 17.5 million metric tons in 2015 (US Geological Survey [USGS], 2017) to 49.5 million metric tons in 2021 (Statistica, 2022), with expansion in mining particularly in tropical and subtropical Asia-Pacific and South America (USGS, 2016). Although not yet in operation, future mining is also likely to include deep-sea mining of Mn nodules on the sea floor in various tropical areas such as the Clarion Clipperton Fracture Zone in the Pacific Ocean (Pettersen & Tawake, 2019).

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Coral reefs throughout the world's tropical oceans are exposed to numerous physical, chemical, and biological stressors (see Meziere et al., 2022). Anthropogenic sources of Mn to the ocean include 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 (Howe et al., 2004; van Hulten et al., 2016; Queiroz et al., 2021).

Despite the importance of tropical coral reefs as foundational habitats, only limited marine toxicity data are available for Mn. Tropical marine species are also under-represented in species sensitivity distributions (SSDs) from which water quality guidelines are derived. There is a need to develop regionally relevant toxicity data for Mn to ensure adequate protection of tropical marine communities.

Corals are classified as cnidarians, a phylum that is one of the most abundant and diverse in tropical marine waters, also including anemones, jellyfish, and hydroids, among other taxa (WoRMs, 2022). Recent studies with cnidarians suggest that adult scleractinan corals are particularly sensitive to Mn (median effect concentrations [EC50s] of 700–860 μg/L, based on dissolved and nominal datasets), even when compared with early life stages of corals (EC50s of 7000–237 000 μg/L, dissolved) and with another cnidarian species (EC50 of 164 000 μg/L, dissolved; Iyagbaye et al., 2022; Stauber et al., 2002; Summer et al., 2019). Reported 48-h EC50 values of 700 μg nominal Mn/L (*Acropora spathulata*) and 860 μg dissolved Mn/L (*Stylophora pistillata*) in static renewal experimental systems suggest that some coral species are sensitive to environmentally relevant Mn concentrations (Stauber et al., 2002; Summer et al., 2019). However, the limitations of these two studies (a nominal, extrapolated EC50 or a value from a commercial in-confidence report) prevented their use in deriving new guideline values for Mn. Given that scleractinian corals are foundation species of coral reef ecosystems, it is imperative that they be included in guideline values developed to ensure protection of economically, ecologically, and culturally valuable coral reef ecosystems (see Deloitte Access Economics, 2017).

The aim of our study was to address data gaps regarding the lack of reliable acute toxicity data on the effects of Mn on scleractinian corals, to help inform the development of marine water quality guideline values for this metal.

MATERIALS AND METHODS

Coral collection and maintenance

Adult coral (*A. muricata*) fragments (five genotypes, 5–10 cm long) were collected from the Great Barrier Reef (Queensland, Australia; Davies Reef, 18°49.826′S, 147°38.192′E), from a depth of between 3 and 6 m. The fragments were glued (XTRA Loctite super glue gel) onto aragonite coral plugs (Ocean Wonders) and transported in 60-L tanks with flowing seawater (5 L/min) to the National Sea Simulator (SeaSim), at the Australian Institute of Marine Science, in Townsville, Queensland. At the SeaSim the corals were held in 200-L semirecirculating flow-through holding systems, supplied with filtered seawater (1 μm) at a rate of 5 L/min, at a temperature of 27 °C. Lighting was provided by Aqua Illumination Hydra LED systems simulating natural daylight cycles (2-h ramp-up, 8 h at 100–150 μmol/m^2/s, 2-h ramp-down, 12 h dark). The corals were fed once a day with a mixture of microalgae (*Nannochloroposis oceania, Pavlova lutheri, Tisochrysis lutea, Chaetoceros muelleri, Chaetoceros calcitrans*, and *Dunaliella* sp.). Corals were acclimated for a period of 4 weeks prior to the beginning of the experiment, and only coral nubbins considered to be healthy with new growth were used in the experiment. Acclimation periods for fragmented corals vary among studies and can range from 4 days to 28 days or more (McLachlan et al., 2020). We selected a period of 4 weeks to ensure that all lesions from the fragmentation process were fully healed, and new polyps were developing. This is in line with Lirman (2000), who showed that moderate lesions healed within 30 days.

Reagents and consumables

Natural filtered (<1 μm) seawater was used as the control and diluent water (Table 1).

All tanks, test vessels, and general glass- and plastic-ware were acid-washed in 10% (v/v) nitric acid (AR grade; Merck), thoroughly rinsed with demineralized water, followed by ultrapure water (Milli-Q®, 18.2 MΩ/cm; Merck), and then rinsed again with seawater prior to use. The flow-through system was flushed with seawater for 48 h prior to test commencement by filling 80-L high-density polyethylene header tanks in the overhead mezzanine laboratory with seawater and pumping the seawater down to treatment vessels through new low-density polyethylene (LDPE) tubing, using peristaltic pumps (Masterflex® L/S Digital Std drive).

TABLE 1: Physicochemical parameters of the seawater used in *Acropora muricata* adult toxicity tests

Parameter	Value
pH	8.1
Salinity (psu)	36–37
Conductivity (mS/cm)	52–54
Dissolved oxygen (% saturation)	98–100
Temperature (°C)	27 ± 0.08 (mean ± 1 SD)
Dissolved organic carbon (mg/L)	0.9–1.2
Dissolved (<0.45 μm) metals (μg/L)[a]	
Al	<1
Cd	<1
Co	<1–2.5
Cr	<1–2.5
Cu	<1
Fe	<1–6
Mn	<0.1–<0.2
Ni	<1.8–<3.7
Pb	<1–2
Zn	<1

[a]Where "<" values are reported, the concentrations were below the detection limit.

Reported as range, unless otherwise indicated; analyzed in samples taken from source seawater (used to prepare treatment solutions in header tanks) on day 0 of each test, except temperature, which was monitored in a water bath throughout the exposures.

Manganese ($MnCl_2{\cdot}4H_2O$; analytical grade; >99% purity) stocks (1000 mg Mn/L) were prepared in ultrapure water on the day of test commencement. Aliquots of this stock were added to 60 L of seawater in the header tanks at nominal concentrations of 0 (Control) and 250, 500, 750, 1000, 2000, 5000, and 10 000 µg Mn/L on the day of test commencement such that the volume of stock added was less than or equal to 1% of the total volume of the treatment, to ensure that physicochemical parameters of treatments were not altered due to stock addition.

Physicochemical parameters of pH, salinity, conductivity, and dissolved oxygen were measured daily using a multiparameter portable meter (HQ40d; Hach) with a PHC301 pH probe, LDO101 dissolved oxygen probe, and CDC401 conductivity probe (all from Hach). Temperature was monitored throughout using temperature sensors (TC Direct, FEP Insulated RTD Pt100 Sensors coupled with a Pt100 4-20mATransmitter) managed by a Siemens S7-1200 micro programmable Logic Controller.

Samples for dissolved organic carbon (DOC) were stored in capped (with Teflon septa) amber glass vials with no headspace at 4 °C. These were analyzed by Australia's National Measurement Institute using a total organic carbon analyzer, method NW_S15 of the American Public Health Association [5310 B; 2005] following a 0.45-µm filtration of sample.

Dissolved metals were operationally defined as 0.45-µm filterable metals. Samples for dissolved metal analyses were collected and filtered through acid-washed (10% HNO_3, AR grade; Merck) 0.45-µm syringe filters into 5-ml polyethylene vials. These samples were preserved by acidifying to 0.2% using nitric acid (0.2% Tracepur HNO_3; Merck). The concentrations of dissolved metals (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were analyzed using inductively coupled plasma–atomic omission spectrometry (ICP–OES; Varian 730 ES). Quality assurance procedures included use of blanks, matrix-matched standards, and drift-correction standards.

Flow-through test design

Two replicate 48-h acute tests were completed under the same lighting and temperature conditions as just described for coral acclimation, without food addition. Test 1 commenced on August 1, 2018, and Test 2 commenced on August 2, 2018. No reference toxicant assay was conducted in parallel because no reference toxicant database existed for this species.

Test chambers and connections to header tanks were similar to those described by Gissi et al. (2019). Briefly, 2.5-L acrylic chambers with acrylic lids were connected to the flow-through delivery system via LDPE tubing (Supporting Information, Figure S1). The tubing was attached to the top of each chamber, where it dripped test solution from the header tanks into the chambers at a rate of 0.17 L/h (two chamber renewals/day). These were maintained at 27 ± 1 °C using water baths, and magnetic stirrers in each chamber ensured even distribution of treatment solution. Solution exited the chambers via tubing at the base of each container.

Two days prior to test commencement, seawater was delivered from header tanks to the treatment vessels via peristaltic pumps and tubing to enable sufficient flushing of the system prior to addition of the corals. During this time, flow rates were optimized to ensure that test chamber renewals occurred twice a day. One day prior to test commencement, the header tanks were refilled with seawater. Coral fragments were also labeled at this time, by randomly selecting adult coral fragments from the five genotypes in acclimation tanks, and attaching preprinted waterproof labels in numerical sequence to each aragonite plug base using Gorilla super glue (example of labels shown in the Supporting Information, Figure S2). Four labeled adult coral fragments on aragonite plugs were then inserted into mounting plates in each of the three replicate chambers/treatment. These were allowed to acclimate for 15 h.

At test commencement, header tanks were refilled to 60 L with filtered seawater, and aliquots of Mn stock were added to the header tanks, mixed well, and pumped through to treatment vessels as just described. Samples for dissolved metal analyses were collected from each header tank at day 0 and each replicate test vessel at six time points (0, 3, 6, 12, 24, and 48 h) to track the Mn concentration in each replicate, as it increased through an initial ramp-up phase (when pumps commenced delivering treatment solution to vessels) to a steady exposure concentration following one complete water change estimated to occur approximately 12 h later (based on rate of 0.17 L/day, to enable two water changes in the 2 L vessel/day).

Physicochemical parameters of pH, salinity, conductivity and dissolved oxygen were measured in each header tank on day 0 and in each replicate chamber at four time points: 0, 3, 24, and 48 h. Samples for DOC were collected from supply seawater on day 0 of each test and in the control header tanks on days 1 and 2 of each test. Physicochemical parameters of pH, salinity, conductivity, and dissolved oxygen were also measured immediately after any removal of dead fragments and manual 50% renewals of fouled treatment water caused by dead fragments.

Visual observations on adult coral health were undertaken during the 48-h exposure to ensure that any dead fragments were removed as quickly as possible to prevent effects from tank fouling on other corals sharing the same tank. At 24 and 48 h, visual observations (healthy, sloughing, or dead) were recorded for each fragment.

Imaging coral fragments

Ex situ photographs. One day prior to test commencement, immediately prior to placing each coral fragment into their randomly allocated test chamber, each coral fragment was photographed at three magnifications (1.4, 4, and 8×) using the same Olympus Tough TG-5 camera on a staged platform with the camera mounted at a set distance of 175 mm from the stage. The same series of ex situ photographs were also taken after a 48-h exposure, following in situ photos. Small plastic containers filled with seawater were used to transport coral fragments between acclimation tanks, the mounted camera stage, and the allocated test chambers. Each fragment was out of seawater for no longer than 1 min during ex situ photographing, and coral fragments were handled by the aragonite base plug to avoid damage to the coral tissue.

In situ photographs. After 48-h exposures, corals were photographed in situ using an Olympus Tough TG-5 with ×8 magnification. A minimum of four in situ photos were taken of each fragment including several at various higher magnifications.

Assessment of coral sloughing

Using both in situ and ex situ images for each coral fragment, the progression of tissue sloughing was assigned a health score: live/healthy = 4; Stage 1 slough = ; Stage 2 slough = 2; Stage 3 slough = 1; dead = 0 (Table 2 and Supporting Information, Table S1). The health score for each of the four coral fragments/tank was averaged so that the maximum health score that any replicate tank could achieve was 4. The health score for each replicate tank was then converted to a percentage score, by dividing the average score/replicate tank by the maximum possible score of four. Each replicate tank percentage score was then normalized to the controls. Levels of acceptable health in the controls were 90% or more healthy adult coral. This criterion was based on typical acceptability criteria for acute vertebrate and invertebrate toxicity tests, for example, those of the US Environmental Protection Agency (USEPA, 2002).

Using National Council for the Social Studies 2023 Ver 23.0.1, the control data from the duplicate tests were tested for assumptions of normality and homogeneity of variance, and there was no significant difference between duplicated test control coral health based on the nonparametric *t*-tests, Mann–Whitney *U* test, or Wilcoxon rank-sum test. Using the same software package, the Kruskal–Wallis multiple comparison test was used to determine which Mn concentrations were significantly different from controls, following tests for normality and equality of variance.

The EC50 concentrations, measured as tissue sloughing (EC50), and the 95% confidence limits were calculated using the drc package in R (Ritz & Streibig, 2005; R Development Core Team, 2018). Effect concentrations were calculated using the time-averaged dissolved Mn concentration (area under the 48-h curve) in each replicate of each treatment. Data were plotted individually for Test 1 and Test 2. A total of seven models were fit to each experiment, and the best model-fit was selected using the Akaike information criterion (see the Supporting Information for the R script).

Assessment of coral bleaching

Ex situ photographs of coral fragments before and following 48-h exposure were analyzed for color intensity using the image analysis software ImageJ (US National Institutes of Health). Assessment of bleaching was done according to the method described by Gillmore et al. (2020), with the modification that the wand feature in ImageJ was used to rapidly trace the coral fragment prior to color intensity analysis. Where partial sloughing (Stages 1, 2, or 3) occurred, traditional tracing techniques in ImageJ were used to select only the portion of the coral fragment with intact tissue for use in color analysis. Fragments without intact tissue were not assessed.

Biochemical analyses

Coral fragments were rinsed with fresh filtered seawater, placed in clean zip-lock bags, and then air blasted to separate the tissue from the skeleton. The tissue slurry was pipetted (using sterile pipettes) into 2-ml cryogenic vials, flash-frozen in liquid nitrogen, and stored at −80 °C. Tissue was collected from four healthy coral fragments prior to test commencement and from all coral fragments with tissue remaining following the 48-h exposure period.

Tissue slurry samples were taken from storage, thawed on ice, and homogenized (Omni tissue homogenizer) in approximately 100 µl buffer (80 mM NaCl, 40 mM Tris base, 8 mM $MgAc_2$, 1 mM ethylenediaminetetraacetic acid [EDTA] at pH 7.5)/10 mg tissue wet weight. Samples were then centrifuged at 5000 *g* for 10 min, and the supernatant was extracted. Total protein content in the supernatant was determined using the Bradford assay. A 5-µl aliquot of protein extract was added to duplicate wells in a 96-well plate, and then 250 µl of Bradford reagent (Sigma-Aldrich; B6916) was added to each well. Following a 10-min incubation at room temperature, the plate was read at 595 nm using a CLARIOstar Plus microplate reader. Total protein was determined using a standard curve prepared from a 7-fold serial dilution of a 1-mg/ml solution of bovine serum albumin (CAS # 9048-48-8) in assay buffer (80 mM NaCl, 40 mM Tris base, 8 mM $MgAc_2$, 1 mM EDTA at pH 7.5).

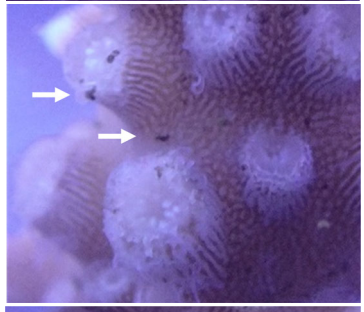
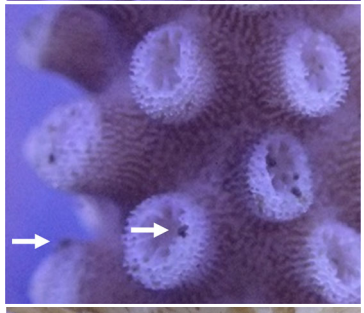
Superoxide dismutase (SOD) activity was analyzed using a 96-well microplate adaption of the Sigma (1999) SOD assay protocol. A 10-µl aliquot of each ultrapure water and protein extract from each fragment was added to 96-well plates; then 280 µl of reaction mix (216 mM potassium phosphate dibasic trihydrate, 10.7 mM EDTA, 1.1 mM cytochrome C, 0.108 mM xanthine at pH 7.8) was added to each well and the microplate was temperature equilibrated to 25 °C using a CLARIOstar Plus microplate reader at 550 nm. Following absorbance stabilization, 10 µl of 0.43 unit/ml xanthine oxidase (in 80 mM NaCl, 40 mM Tris base, 8 mM $MgAc_2$, 1 mM EDTA at pH 7.5) was added to each well using a multichannel pipette, and absorbance was immediately recorded at 550 nm and at 60-s intervals for 5 min. The change in absorbance between each 60-s interval was then used to determine an overall mean change in absorbance in each sample. The SOD activity is inversely determined through comparing the change in absorbance between the samples and an uninhibited control reaction, and results in a percentage inhibition value. This percentage inhibition value was subsequently converted to SOD activity whereby 50% inhibition is related to 1 unit of SOD activity (Sigma, 1999). By standardizing the sample activity to the total protein quantified in the Bradford assay, SOD activity/mg of protein was determined.

RESULTS

Physical and chemical measurements

One sample of seawater was taken from each of the following, for both Tests 1 and 2: source water on day 0 (used to prepare treatments): control header tank on day 1 and control

TABLE 2: Health scoring criteria for *Acropora muricata* with example photographs (see Supporting Information, Table S1 for more examples)

Criterion	Description	Example	Score
Healthy	• Tissue and *Symbiodinium* sp. clearly intact giving a "pattern" (often striped) to the tissue • Polyp tips have a "fleshy" look • No sign of bleaching • Tentacles often visible		4
Stage 1 slough	• Some polyp tips showing sloughing where tissue has a "ragged" appearance rather than "fleshy" when in situ • Clearly some healthy poly tips visible • *Symbiodinium* sp. seem intact and "pattern" evident • Sublethal		3
Stage 2 slough	• Throughout fragment polyp tips sloughing, tissue "ragged" appearance rather than "fleshy" at tip when in situ • *Symbiodinium* sp. seem intact and "pattern" evident, clumps (e.g., as indicated by arrows) may be visible • May see retracted tentacles • Likely lethal		2
Stage 3 slough	• Advance sloughing • Clear evidence that tissue is compromised • *Symbiodinium* sp. clumps (e.g., as indicated by arrows) often visible "pattern" compromised in places • Skeleton on polyp tips exposed • Recovery not possible • Tentacles not visible		1
Dead	• Obvious tissue disintegration • White skeleton clearly visible • *Symbiodinium* sp. pattern in tissue only slightly evident, or not evident at all • Recovery not possible		0

header tank on day 2. The DOC values of the seawater in the source water on day 0 and the control header tank on days 1 and 2 were 1.2, 1.4, and 1.2 mg/L respectively, for Test 1, and 0.9, 1.2, and 1.1 mg/L, respectively, for Test 2.

There are no acceptability criteria for coral toxicity tests with respect to physicochemical conditions. The USEPA (2002) guidance for chronic marine toxicity tests with their select species indicate that in general, dissolved oxygen should be >4 mg/L. Gissi et al. (2019) reported normal ranges for pH, salinity, and dissolved oxygen in 96-h flow-through exposures with *A. muricata* (using the same flow-through design as our own study) to be 8.1 ± 0.2, 35 ± 1 psu and >80% saturation, respectively. In our 48-h exposure, the salinity and conductivity remained stable throughout all replicates in all tests at 35–36 psu and 53–55 mS/cm, respectively. The pH ranged from 8.1–8.2, and the dissolved oxygen ranged from 7.5–8.8 mg/L (88%–107% saturation) across all replicates and treatments in both tests on day 0 (Supporting Information, Tables S2–S4). The dissolved oxygen in controls remained well above 4 mg/L throughout the tests, ranging from 5.6 to 9.0 mg/L. Similarly, the pH in controls remained within the normal range of 8.1 ± 0.1 throughout both tests. After 24 and 48 h, decreases in pH and dissolved oxygen were observed in tanks where tissue loss or mortality was observed (Supporting Information, Tables S2–S4). After removal of dead fragments at 24 h, syringe-removal of mucus, and 50% treatment renewals, physicochemical parameters were measured again, but did not always return to the range of those in controls and other treatments, that is, where there was extensive tissue loss/mortality, dissolved oxygen and pH remained lower than controls (Supporting Information, Tables S2 and S3). The lowest pH observed following the 50% renewal was 7.8, which is within the normal range found in coral reef shorelines (see Santos et al., 2011). In treatments where the dissolved oxygen remained low, it is possible that coral mortality was partly due to additional stress of low oxygen conditions. Note, however, that several treatments with oxygen concentrations similar to controls (7–8 mg/L) contained fragments that were unhealthy or dying, that is, whereas low dissolved oxygen may have contributed to toxicity in the highest test concentrations, impaired health detected at lower concentrations was more likely due to Mn exposure alone. It is noted, however, that future studies should consider incorporating larger tanks, with higher flow rates, to ensure dissolved oxygen is maintained at acceptable levels.

The limit of detection for Mn using ICP–OES ranged from 0.1 to 0.2 µg/L. The detection limits for other metals were 1–2 µg/L (Al); 1–1.5 µg/L (Cd); 1 µg/L (Cr); 1 µg/L (Co); 1–2.5 µg/L (Cu); 1 µg/L (Fe); 1–1.5 µg/L (Ni); 5 µg/L (Pb); and 1 µg/L (Zn).

The measured dissolved concentrations of Mn gradually increased in treatment vessels following commencement of the ramp-up phase of exposure, with 19%–34% of the nominal Mn concentration detected after 3 h. This increased to 50%–57% of nominal after 24 h, and 84%–93% of nominal by 48 h, similar to those in the header tanks, which were 87%–90% of the nominal concentration, that is, by 48 h, the concentration of dissolved Mn in the test containers was the same as that in the header tanks (Supporting Information, Table S5). The time-averaged concentrations of dissolved Mn in all treatments were 63%–68% of the nominal Mn concentration (Table 3).

The concentrations of other dissolved metals detected in header tanks and treatment vessels were mostly close to or below detection level (all dissolved metal measurements provided in the Supporting Information).

Coral sloughing

The health scores for control adults in Tests 1 and 2 were 100% ± 0% and 92% ± 14% (mean ± standard deviation, $n = 3$),

TABLE 3: Forty-eight hour concentration–response data for adult coral (*Acropora muricata*) health based on tissue sloughing.

		Dissolved Mn (µg/L)		Coral health (%)		Coral health (% control)		
Test no.	Nominal Mn (µg/L)	Time-averaged	SD	Mean	SD	Mean	SD	N
1	0	<LOD[a]	N/A	100	0	100	0	3
1	250	160	7	92	14	92	14	3
1	500	330	8	85[b]	25	85	25	3
1	750	490	12	69[b]	49	69	49	3
1	1000	660	27	79[b]	7	79	7	3
1	2000	1300	38	29[b]	26	29[c]	26	3
1	5000	3100	140	0	0	0[c]	0	3
1	10 000	6600	420	0	0	0[c]	0	3
2	0	<LOD	N/A	92[b]	14	100	16	3
2	250	160	12	92	14	100	16	3
2	500	330	10	67[b]	58	73	63	3
2	750	490	13	77[b]	25	84	28	3
2	1000	630	63	44[a]	44	48	48	3
2	2000	1300	15	33[b]	32	36	35	3
2	5000	3400	34	0	0	0[c]	0	3
2	10 000	6600	53	0	0	0[c]	0	3

[a]Limits of detection (LODs) were between 0.1 and 0.2 µg/L.
[b]Ejected mesenterial filaments (Supporting Information, Figure S3) observed on one or more fragments.
[c]Significantly different from control, using the Kruskal–Wallis multiple comparison test.
N/A = not available.

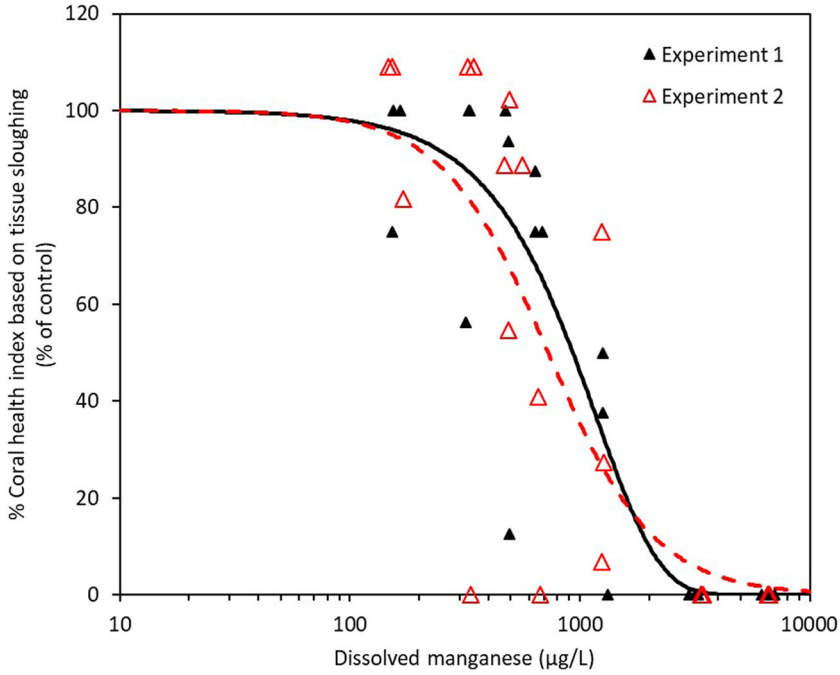


FIGURE 1: Concentration–response plots of 48-h coral health based on tissue sloughing and using the measured dissolved time-averaged manganese concentration for each replicate tank treatment in two experiments. The black solid and red dashed lines are the modeled responses in Tests 1 and 2, respectively. Concentration–response plots were generated in the drc package for R (Ritz & Streibig, 2005; Supporting Information) using the 4-parameter Weibull model for Experiment 1, and the 3-parameter log–logistic model for Experiment 2. Each point represents the mean response for each replicate treatment vessel ($n = 3$).

respectively, and therefore met the criterion for acceptability of 90% or more healthy adult coral (Table 3). The survival of control adult coral in Tests 1 and 2 was 100% and 92%, respectively. Within 24 h, extensive mucus production was observed in tanks containing fragments with advanced sloughing and/or that were dead. Therefore, to avoid tank fouling, at 24 h, dead fragments were removed, and mucus that was floating on the surface of the water was removed by syringe, followed by 50% water renewals. Ejected mesenterial filaments (MFs) were also observed on some coral fragments (Supporting Information, Figure S3). The implication of ejected MFs for coral health is not known. They can be a response to stress (Lewis, 2018) but are also used by corals to cause damage to surrounding organisms as a defence mechanism (Nugues et al., 2004) and may also act as a cleaning mechanism to clear substrate prior to growth and expansion (Roff et al., 2008). Several MFs were also detected in two control fragments after 48 h of exposure. Therefore, the presence of MFs was not used as an index of coral health. Due to wide variability in the health score of corals across treatments, only the highest test concentrations (1300, 3100, and 6600 µg dissolved time-averaged Mn/L in Test 1 and 3400 and 6600 µg dissolved time-averaged Mn/L in Test 2) were significantly different from controls (Table 3).

There were no marked differences between the time-averaged concentrations of the same treatments in different tests. A clear concentration–response relationship was observed for the separate experiments (Figure 1).

For use in an SSD to derive a default guideline value, the acute EC10 value is not recommended for conversion to chronic values. Instead, the acute EC50 value is converted by dividing by a default acute-to-chronic ratio of 10 (Warne et al., 2018). The 48-h acute EC50 values for coral health (based on tissue sloughing) for Test 1 and Test 2 were 933 and 728 µg dissolved Mn/L, respectively (Table 4 and Figure 1). The geometric mean of these two values is 824 µg dissolved Mn/L. This converts to an estimated chronic value of 82 µg dissolved Mn/L.

Coral bleaching

All intact coral tissue remaining following the 48-h exposure period had darkened compared with day 0, with color intensity >100% of the original initial color in all treatments including the controls (Figure 2). No bleaching was observed in corals following exposure to Mn. Conversely, and similar to the controls, an increase in color intensity was observed in most Mn-enriched treatments. This increase was significantly higher than in controls at the lowest test concentration of 160 µg time-averaged dissolved Mn/L in Test 2.

TABLE 4: Effect concentrations (EC10 and EC50) for 48-h adult coral (*Acropora muricata*) health (based on tissue sloughing) when exposed to dissolved manganese

Test	EC10 (µg/L)[a]	EC50 (µg/L)[a]
Test 1	280 (57–510)	930 (660–1200)
Test 2	230 (19–440)	730 (400–1100)
Geometric mean	250	820

[a]Concentration of the sample to cause a 10% (EC10) or 50% (EC50) effect; values in parentheses are 95% confidence limits; rounded to two significant figures.

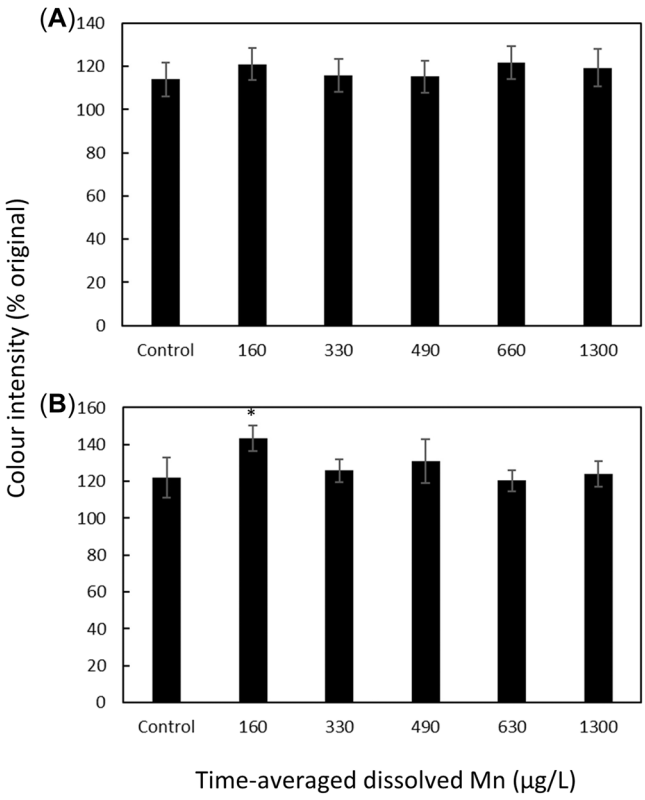


FIGURE 2: Color intensity change in tissue following 48-h exposure to dissolved (time-averaged) manganese in (**A**) Test 1 and (**B**) Test 2. Asterisk indicates significantly difference from all other treatments.

Coral SOD activity

There were no consistent trends with respect to SOD activity in coral tissue following exposure to Mn across both tests (Supporting Information, Figure S4). There appeared to be a general trend of increasing SOD with increasing concentration of Mn in Test 1, but this was not evident in Test 2. There was a large variability in SOD activity in coral tissue following exposure to Mn, which was thought to possibly be due to the variability in coral response within each exposure concentration, with some treatments containing fragments at differing levels of health. However, when the SOD activity was replotted as a function of health score, there were still no consistent patterns across both tests (Supporting Information, Figure S4).

DISCUSSION

Using carefully designed, replicated flow-through experiments, with measurements of dissolved Mn throughout exposures, we showed that within a 48-h exposure, dissolved Mn caused tissue sloughing in adult coral *A. muricata* fragments, with an acute time-averaged EC10 of 250 µg/L and an EC50 of 820 µg/L (two significant figures). Only two previous studies of Mn toxicity to adult scleractinian corals exist. Stauber et al. (2002) first reported tissue sloughing in response to similar low concentrations of dissolved (<0.45 µm) Mn in *S. pistillata*, (48-h EC50 860 µg/L). These data were cited in Australia and New Zealand Environment and Conservation Council/Agricultural and Resource Management Council of Australia and New Zealand (2000); however, the original data were restricted within a commercial in-confidence report. A second study by Summer et al. (2019) using limited replicates and only nominal concentrations found tissue sloughing of *A. spathulata* to occur in all concentrations tested (1000–50 000 µg/L, nominal). The present study provides the first definitive published data to support the hypothesis put forward by these two earlier studies that adult scleractinian corals are particularly sensitive to Mn. Acute toxicity to Mn was observed at dissolved concentrations well below those reported to cause acute and chronic toxicity to other marine species (acute EC50 26 000 µg/L; chronic no-observed-effect concentrations 1000–125 000 µg/L) and to other coral life stages (EC10s 4000–43 000 µg/L, EC50s 7000–237 000 µg/L; Summer et al., 2019).

Early life stages are usually assumed to be more sensitive than adults to environmental contaminants and stressors (Mohammed, 2013), and as a result there is a preference to use early life stages in toxicity testing programs. Indeed, based on the literature available for organic contaminants (dispersants, pesticides, and oils), early life stages (larvae and gametes) of corals are generally more sensitive than adults (Markey et al., 2007; Negri et al., 2018; Turner & Renegar, 2017). However, for metals, the relationship between toxicity and life stage for corals is not as clearly defined, and there are some species-specific sensitivity differences to some metals that make generalizations difficult. Also, toxicity data across multiple coral life stages are only available for Cu, Pb, NI, Cd, and Mn.

Copper is toxic to all life stages of scleractinian corals at concentrations from 11 to 145 µg Cu/L, with no key life stage seeming to be more or less sensitive (Gissi et al., 2017, 2019; Hedouin et al., 2016; Mitchelmore et al., 2007; Reichelt-Brushett & Harrison, 2000, 2004, 2005). For Pb, coral toxicity data are limited to four species in three studies. Adult *Pocillopora damicornis* (median lethal concentrations [LC50s] of 477–724 µg Pb/L) are approximately threefold more sensitive than gametes of *Acropora longicyathus, Acropora tenuis*, and *Goniastrea aspera* (EC50s for fertilization success from 1453 to 2467 µg Pb/L; Hedouin et al., 2016; Reichelt-Brushett & Harrison, 2004, 2005). However, for larvae, there is a 20-fold difference between the 72-h LC50 for *G. aspera* (9890 µg Pb/L) and the 96-h LC50 for *P. damicornis* (462–681 µg Pb/L across temperatures of 27–30 °C (Hedouin et al., 2016; Reichelt-Brushett & Harrison, 2004). It is important to note that *P. damicornis* is a brooding coral species that release fully formed larvae, whereas *G. aspera* spawn gametes that fertilize externally. These data suggest that *G. aspera* larvae are less sensitive to Pb than fertilization success of gametes, and that both larvae and gametes of this species are generally less sensitive to Pb than adults of other species, whereas for *P. damicornis*, larvae are of similar sensitivity to Pb as adults.

Nickel is between 2- and 45-fold more toxic to adult corals than to early life stages with effects on the algal symbionts. Concentrations of 470 and 200 µg Ni/L caused bleaching (but not mortality) to adult *A. muricata* following 4- and 7-day

exposures, respectively (Gillmore et al., 2020; Gissi et al., 2019). However, effects on larval development and fertilization have not been observed at concentrations below 1000 μg/L on species tested (see Gissi et al., 2017; Reichelt-Brushett & Harrison, 2005). The EC50 for *P. damicornis* larval survival is as high as 9000 μg Ni/L (Gissi et al., 2017).

Cadmium toxicity data for corals is limited; however, 50 μg Cd/L caused sloughing and mortality to adult *P. damicornis* in the absence of bleaching (Mitchelmore et al., 2007), whereaas concentrations as high as 200 and 1000 μg Cd/L did not reduce fertilization success in gametes of *G. aspera* and *Oxypora lacera*, respectively (Reichelt-Brushett & Harrison, 1999). This indicates that, similar to Mn, Cd is more toxic to adults than to early life stages through effects directly on the host, not through algal symbionts.

Collectively, these data show that increased sensitivity of adult corals compared with early life stages with respect to metals is not uncommon. However, for Mn, the magnitude of this difference is larger than for any other metal. The EC50 values based on sloughing of 700–860 μg Mn/L (present study; Stauber et al., 2002; Summer et al., 2019) were up to 340-fold lower and 10–40-fold lower than those found by Summer et al. (2019) for coral gamete fertilization (EC50s 164 000–237 000 μg/L), and larval mortality (EC50s of 7000–28 000 μg Mn/L), respectively.

Sensitivity does not always correlate with life stage, with examples evident for fish (Fort et al., 2004), amphibians (Shen et al., 2020), and a range of invertebrates (Holan et al., 2018), including corals (He et al., 2019). Instead, it is more likely to depend on the speciation in solution, route of uptake, mode of toxicity, and physiology of the given life stage. For example, Diuron, a photosystem II herbicide, was not toxic to *P. damicornis* larval development (metamorphosis), fertilization, or recruit survival at 1000 μg/L, but caused nonrecoverable tissue retraction in recruits at 100 μg/L, bleaching of adults and recruits at 10 μg/L, and irreversible bleaching in recruits at 1 μg/L (Negri et al., 2005). These results were explained by the action of Diuron on photosynthesis of algal symbionts, which older life stages of *P. damicornus* rely on for energy, but gametes and larvae do not require for fertilizsation or larval development.

The mechanism of Mn toxicity to adult scleractinian corals has not yet been identified. Studies on aquatic organisms have shown that exposure to environmentally relevant concentrations of Mn causes oxidative stress (Hernroth et al., 2020) and can enhance production of reactive oxygen species (ROS) predominantly in the mitochondrial matrix of cells (Martinez-Finley et al., 2013). Elevated ROS can in turn cause damage to DNA and other intracellular components, potentially leading to apoptosis (Hernroth et al., 2020). We therefore hypothesized that coral tissue necrosis could be due to excessive ROS being generated at the coral surface possibly through reactions of Mn with free radicals or possibly through increased photosynthesis of algal symbionts using excess Mn as a nutrient (Biscere et al., 2018). The latter explanation supports our finding that Mn did not seem to interfere with the symbiotic relationship, because we did not find evidence of bleaching. Antioxidant enzymes such as SOD regulate ROS production and are therefore used as biomarkers for oxidative stress. However, we did not observe any consistent trend with respect to SOD activity in coral tissue following exposure to Mn. The SOD enzyme is just one type within a range of antioxidant enzymes that corals produce to scavenge excess ROS. Shinzato et al. (2012) identified seven SOD proteins, along with nine catalase proteins, and a host of other oxidative stress proteins in the genome of closely related *Acropora digitifera*. It is possible that use of additional biomarkers, for example, to detect catalase activity, or metabolomics and transcriptomics would help us to understand the relationship between Mn exposure and ROS production.

Although limited to just two studies, there is some evidence to suggest that closely related noncalcifying cnidarians appear to be less sensitive to Mn than scleractinian corals. The anemone *Exaiptasia pallida* survived after a 12-day exposure to 54 000 μg dissolved Mn/L (Summer et al., 2019), and up to 87 000 μg dissolved Mn/L following 24 days exposure; however, stress responses of tentacle retraction were observed at concentrations as low as 460 μg dissolved Mn/L (Iyagbaye et al., 2022). Iyagbaye et al. (2022) also showed that *Symbiodinium* spp. played a role in Mn uptake and depuration from *E. pallida*. Furthermore, no substantial impact on *Symbiodinium* spp. density was noted between the controls and treatments, which was also observed for *A. muricata* symbionts via color intensity (Figure 2) in the present study. Nevertheless, the severe sloughing response observed for *A. muricata*, *S. pistillata*, and *A. spathulata* at concentrations below 1000 μg dissolved Mn/L (present study; Stauber et al., 2002; Summer et al., 2019) has yet to be observed for noncalcifying cnidarians, suggesting that the sensitivity of scleractinian corals to Mn may be related to interferences with the skeletal matrix. For example, Summer et al. (2019) suggested possible interference of Mn^{2+} with Ca^{2+} transport at calcifying sites, or substitution of Mn^{2+} for Ca^{2+} in the coral matrix during skeletogenesis. It is also possible that Mn^{2+} is substituted for Ca^{2+} within skeletal organic matrix proteins. Cadherin is a cell–cell adhesion protein found in many vertebrates and recently identified in the skeletal proteome of a related scleractinian coral *A. digitifera* (Takeuchi et al., 2016). The adhesion mechanism in cadherin is calcium dependent because Ca^{2+} ions bind together extracellular repeats (Gumbiner, 2005), and this was further proved by Domart-Coulon et al. (2004), who induced coral to slough in Ca-free artificial seawater.

The mode of toxicity of Mn may also be through increased susceptibility to infection. Exposure to dissolved Mn suppresses the immune system of aquatic organisms, thereby reducing the animal's bacteriostatic capacity, and increasing its susceptibility to infections (Hernroth et al., 2020). Coral tissue sloughing and necrosis have been observed as a result of pathogen infections such as the "stony coral tissue loss disease" that has ravaged corals throughout the Florida Keys (USA; Aeby et al., 2019) and through associations with *Vibrio* species (Munn, 2015). However, given the short exposure period in the present study (48 h), it is unclear whether this would provide sufficient time for infection to occur.

Tissue sloughing in adult *Pocillopora verrucosa* occurred following 14-day exposure to excess total organic nitrogen and

was accompanied by an increase in the density and a shift in the community structure of associated *Symbiodinium* (Pogoreutz et al., 2018). After a 7-day exposure, fragments under excess total organic nitrogen were visibly darker in color. Minor (<10%) tissue sloughing first appeared after 13 days exposure and rapidly advanced over the next day to over 90% tissue loss. The authors attributed tissue sloughing to reduced carbon translocation by the overgrown Clade A *Symbiodinium*. Manganese is an essential nutrient for microalgae and when enriched in media, can stimulate algal growth (Ciurli et al., 2021). Excess Mn may have caused a similar shift in the *Symbiodinium* community on *A. muricata*. All coral fragments with intact tissue following Mn exposure had darkened tissues (including controls); however, fragments in some Mn exposure concentrations were darker than controls, which was significant at 160 µg Mn/L in Test 2. However, as *Symbiodinium* densities and their community structure were not determined in our study, this cannot be confirmed.

Another possibility is that the coral's physical defence system triggered an irreversible over-response to Mn that resulted in tissue necrosis. Coral mucus has a variety of roles in coral health, one of which is a defence mechanism against physical and chemical stressors. Coral mucus is thought to act as a physical barrier, protecting coral tissue from sedimentation and desiccation; to provide an avenue for accumulated contaminants to be released back into the water column (e.g., for quenching of harmful oxygen radicals), and it contains powerful antibiotic and UV-quenching properties to protect corals from bacterial infection and UV radiation (Brown & Bythell, 2005). Following exposure of adult *Porites divaricata* to oil, Renegar et al. (2017) observed an apparent exhaustion of mucus production, coupled with an increased granular amoebocyte area in the epidermis, which led to tissue necrosis. So, it is possible that following Mn exposure, the mucus production by *A. muricata* in our study was the precursor leading to sloughing. Certainly, mucus production was observed wherever unhealthy fragments were detected. However, this does not explain why adult scleractinian corals, more than any other species, would react to such relatively low concentrations to Mn, an essential metal at nanomolar concentrations required for coral health (Biscere et al., 2018).

With the exception of Cu, the concentrations of metals that are toxic to any life stage of coral are generally above environmentally relevant concentrations. The EC10 for adult *A. muricata* in the present study was 250 µg dissolved Mn/L. Dissolved Mn has been detected at concentrations up to 100 µg/L in marine waters affected by anthropogenic inputs (Hatje et al., 2003), and up to 500 µg/L in anaerobic layers of open seawater (World Health Organization, 2004). Mining activities have the potential to introduce mg/L concentrations of Mn to receiving marine waters (Apte et al., 2007; Nieto et al., 2007). Therefore, there is a potential for Mn to enter the water column at concentrations that will have acute toxicity on corals. Furthermore, we observed acute toxicity within just 48 h, but the chronic toxicity of Mn to adult corals over longer exposure periods is not known, and is anticipated to occur at much lower Mn concentrations. Future studies assessing chronic toxicity are required to confirm this. Future studies should also consider using higher flow-through rates, and potentially larger tanks than those used in our study, to ensure better clearance of fouled water where sloughing and mortalities are observed and ensuring that pH and dissolved oxygen remain consistent throughout all treatments.

The current Mn guideline value for Australian and New Zealand marine waters of 80 µg/L (Australian and New Zealand Guidelines, 2018) is described as being of unknown reliability due to the use of an assessment factor of 200 applied to the lowest available acute EC50 for a mollusc. In the absence of chronic toxicity data for scleractinian adult corals, the current high-quality acute data will contribute to more robust guidelines that will protect the most sensitive life stage of coral.

Our findings confirm that Mn is toxic to adult scleractinian corals at concentrations well below those of many other marine species, and well below those of scleractinian early life stages. We propose several theories for this heightened sensitivity, but further research is required to identify the modes of action of Mn to scleractinian corals. Such studies would benefit greatly from a range of omics approaches. For example, we theorized that tissue sloughing could be due to infection with pathogens or shifts in the microbiome population and community structure (genomics); interference with skeletogenesis and/or skeletal proteins (proteomics); or an overactive defence response to physical and chemical stress (metabolomics, transcriptomics).

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