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Acute and chronic toxicity of manganese to tropical adult coral (*Acropora millepora*) to support the derivation of marine manganese water quality guideline values

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ABSTRACT

Adult corals are among the most sensitive marine organisms to dissolved manganese and experience tissue sloughing without bleaching (i.e., no loss of *Symbiodinium* spp.) but there are no chronic toxicity data for this sensitive endpoint. We exposed adult *Acropora millepora* to manganese in 2-d acute and 14-d chronic experiments using tissue sloughing as the toxicity endpoint. The acute tissue sloughing median effect concentration (EC50) was 2560 μg Mn/L. There was no chronic toxicity to *A. millepora* at concentrations up to and including the highest concentration of 1090 μg Mn/L i.e., the chronic no observed effect concentration (NOEC). A coral-specific acute-to-chronic ratio (ACR) (EC50/NOEC) of 2.3 was derived. These data were combined with chronic toxicity data for other marine organisms in a species sensitivity distribution (SSD). Marine manganese guidelines were 190, 300, 390 and 570 μg Mn/L to provide long-term protection of 99, 95, 90, and 80 % of marine species, respectively.

1. Introduction

Manganese (Mn) is a critical element used primarily as an alloy with iron to increase the hardness of steel but also for renewable energy storage in dry cell batteries, as well as in paints, inks, glass, ceramics, fireworks and fertilisers (Summerfield, 2021; WHO, 2004). As the renewable energy economy and global demand grows for manganese and other associated critical elements, the increase in processing manganese-containing ores will inevitably result in the increased release of dissolved manganese into marine ecosystems (Miller et al., 2018; Toro et al., 2020). Adult corals have been shown to be among the most sensitive species to dissolved manganese (Binet et al., 2023b; Stauber et al., 2002; Summer et al., 2019) and are a high priority for environmental protection because of the ecosystem services they provide and their economic significance (Boakes et al., 2022; Costanza et al., 1997; Deloitte Access Economics, 2017). The derivation of long-term water quality guideline values (GVs) is warranted to provide robust benchmarks for assessing potential adverse effects of dissolved manganese on marine biota such as coral.

Dissolved manganese (operationally defined as the <0.45 μm filtered fraction) enters oceans naturally from deposition and dissolution of atmospheric manganese-particulates in the form of aeolian dust, volcanic emissions, terrestrial erosion and runoff as well as fluvial discharges and release from acid-sulfate soils (Van Hulten et al., 2017; WHO, 2004). Anthropogenic sources of manganese include municipal wastewater discharges, wastes from coal and mineral mining and processing, emissions from steel and iron production, and combustion of fossil fuels (WHO, 2004).

Corals exist in harbours, mangroves, coasts, and oceanic locations in near-surface and deep-sea habitats where exposure to dissolved manganese can occur. There is a decreasing horizontal gradient in the natural background dissolved manganese concentration of surface waters from coastal (0.082–0.38 μg/L) to open ocean (0.004–0.27 μg/L) locations due to terrestrial influences (Table S1). There is also a decreasing

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vertical gradient in concentration from surface to deep-seawaters due to manganese adsorption to particulates, and microbially-catalysed oxidation of dissolved manganese to solid manganese oxide phases (Hansel, 2017; Oldham et al., 2020; Van Hulten et al., 2017). However, if anoxic conditions occur at the sediment/water interface of coastal mangrove or deep-sea sediments, reducing-bacteria catalyse the reduction of manganese oxides resulting in a flux of dissolved manganese from the sediment into the water column at concentrations that are orders of magnitude higher than surrounding background levels (Van Hulten et al., 2017), (Table S1). In addition, deep-sea hydrothermal vent emissions can also increase the dissolved manganese concentration of surrounding waters for several kilometres (Van Hulten et al., 2017). Anthropogenic activities have increased the dissolved manganese level above background concentrations to those currently ranging from 0.13 to 100 μg Mn/L in ports and harbours, and can be as high as 582 ± 626 μg Mn/L when there is a catastrophic failure of a tailings dam (Queiroz et al., 2021) (Table S1).

Of the >11 possible oxidation states of manganese, Mn(II), Mn(III) and Mn(IV) are most relevant to marine waters (Oldham et al., 2017; Oldham et al., 2020; Tebo et al., 2004; WHO, 2004). The dominant form of manganese is dissolved Mn(II) as $Mn(H_2O)_6^{2+}$, but low concentrations of inorganic complexes with chloride, sulfate, bicarbonate or organic ligands also exist (Hansel, 2017). Organic complexes of Mn(III)-ligand can remain stable and dominate the dissolved phase, although in the absence of ligands, Mn(III) disproportionates rapidly to dissolved Mn(II) and colloidal/solid Mn(III/IV) phases (Hansel, 2017; Oldham et al., 2017). Solid Mn(IV) as MnO_2 is the thermodynamically favoured form of manganese under oxic conditions, however oxidation of Mn(II) by abiotic processes is extremely slow and the presence of sunlight inhibits oxidizing-bacteria so that dissolved Mn(II) dominates instead (Hansel, 2017; Sunda, 2012; Tebo et al., 2004).

Dissolved Mn(II) is the most bioavailable form of manganese in marine waters and therefore more toxic than colloidal and solid Mn(III/IV) forms (WHO, 2004). The environmental chemistry and hence toxicity of manganese in marine waters is largely controlled by redox conditions, pH, sunlight, microbial activity and organic matter (indirectly by catalysing microbial activity) (WHO, 2004). Because manganese is an essential element, organisms can regulate excess internal concentrations to a species-specific threshold before toxicity occurs. Corals accumulate manganese at higher concentrations in the symbiotic dinoflagellates (*Symbiodinium* spp.) than the host tissue and skeleton which suggests that the *Symbiodinium* spp. are involved in regulating manganese in the host coral (Metian et al., 2015; Reichelt-Brushett and McOrist, 2003). The toxicity of manganese to marine biota generally occurs at dissolved concentrations above 1000 μg Mn/L (WHO, 2004), which is usually well in excess of environmental concentrations. One of the exceptions to this is adult corals.

Three adult coral species (*Acropora spathulata*, *Acropora muricata*, and *Stylophora pistillata*) have acute 48-h median effect concentrations (EC50) of 700–933 μg Mn/L for effects on tissue sloughing (Binet et al., 2023b; Stauber et al., 2002; Summer et al., 2019). When these are converted to estimated chronic toxicity values with a default acute-to-chronic ratio (ACR) of 10, the toxicity values become 70–93 μg Mn/L which is within the concentration range of marine waters currently contaminated by manganese (Table S1). Tissue sloughing involves the disconnection of coral tissue from the skeleton without causing bleaching, i.e., without loss of the *Symbiodinium* spp. (Binet et al., 2023b; Stauber et al., 2002). As the area of tissue sloughing increases, a tipping point is reached where the coral does not recover and eventually it dies. Acute 48-h tissue sloughing in adult coral is more sensitive than biological endpoints of early life stages such as acute 24-h larval mortality (EC50 value of 7000 μg Mn/L) and chronic 5.5-h gamete fertilisation success (EC50 value of 164,000 μg Mn/L) (Binet et al., 2023b; Summer et al., 2019). There are currently no chronic toxicity data for the sensitive adult coral tissue sloughing endpoint that can be used to derive long-term protective water quality GVs.

The mechanism of manganese toxicity that causes tissue sloughing in adult coral is unknown. However, Binet et al. (2023b) and Summer et al. (2019) hypothesized that oxidative stress, immunosuppression, calcium interference and excess mucus production could all contribute to the mechanism of toxicity. Mechanistic studies of manganese toxicity to other biota have so far been conducted at environmentally unrealistically high dissolved concentrations making interpretation of the data problematic (Hernroth et al., 2020; Martin et al., 2008; Martinez-Finley et al., 2013; Oweson et al., 2006; Pinsino et al., 2011; Sköld et al., 2015). In contrast, low-level environmentally relevant enrichment of dissolved manganese (4 μg Mn/L) has been shown to benefit adult coral (*S. pistillata*) by increasing resistance to heat stress-induced bleaching (Biscéré et al., 2018; Montalbetti et al., 2021). This emphasizes how manganese is essential and beneficial to adult coral and their symbionts at low concentrations but becomes toxic when a certain concentration threshold is exceeded. Therefore, further research is needed on adult coral species to determine the range in species sensitivity to manganese and to fill the chronic toxicity data gap for deriving long-term protective water quality GVs.

The Australian and New Zealand guidelines for fresh and marine water quality focus on long-term protection of marine ecosystems, and are preferably based on high quality chronic toxicity data (ANZG, 2018; Batley et al., 2018; Warne et al., 2018). Due to insufficient chronic manganese toxicity data, Australia and New Zealand currently have a low-reliability interim working level for marine waters based on five data points from three taxonomic groups, comprising one crustacean (acute), two bivalves (acute), and two diatom (chronic) values (ANZG, 2018). The lowest toxicity value was for the oyster (*Crassostrea virginica*, 48-h LC50 = 16,000 μg/L, Calabrese et al. (1973)) which was divided by an assessment factor of 200 (for an essential metal) to give a low-reliability interim working level of 80 μg Mn/L for marine waters (ANZG, 2018). For comparison, a 95 % species protection level marine manganese GV of 300 μg/L was derived by WHO (2004) using a species sensitivity distribution (SSD) that combined acute and chronic data, and applied assessment factors of 5 or 10 to chronic EC50 or acute LC50 values, respectively, to estimate chronic no-observed-effect concentrations (NOECs). Levy et al. (2004) used toxicity data from five Australian species (three chronic NOEC values and two acute NOEC values converted to estimated chronic values using an acute-to-chronic ratio of 3.1) including a fish, as well as the WHO (2004) data in an SSD to derive a site-specific GV of 660 μg/L for 95 % species protection. Stauber (2006) combined the WHO (2004) and Levy et al. (2004) data (using an acute-to-chronic ratio of 12.4 instead of 3.1) as well as a sensitive adult coral (Stauber et al., 2002), in an SSD to derive a site-specific manganese GV of 140 μg/L at the 95 % species protection level. The GVs derived by WHO (2004), (Levy et al., 2004) and Stauber (2006) are disparate and two to eight times higher than the ANZG (2018) low-reliability interim working level suggesting that the ANZG (2018) value is overly conservative. Acute and chronic marine manganese toxicity data have become available in the literature in recent years which can now be used to update the low-reliability interim working level, however, there are still no chronic toxicity data for adult corals.

The current study aimed to provide the first acute and chronic manganese toxicity data for the sensitive biological endpoint of tissue sloughing for the adult reef-building scleractinian coral, *Acropora millepora*, and an ACR that can be used to convert existing acute manganese toxicity data for other coral species to estimate chronic data. This will fill a vital knowledge gap regarding the variation in toxicity of manganese to adult coral species and optimise the use of acute toxicity data for adult corals. We aimed to collate, review and assess chronic manganese toxicity data for other marine biota from the literature and to use these with the chronic toxicity data for *A. millepora*, and estimate chronic toxicity data for other acute coral endpoints to provide robust marine water quality GVs with varying levels of long-term protection for marine ecosystems.

2. Methods

2.1. General experimental methods

All experimental work was undertaken at the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS), Townsville, Queensland, Australia (Fig. S1). All tanks and flow lines were cleaned with a series of bleach, acid and filtered (<1 μm) natural seawater (FSW) flushes prior to experimentation. Treatment tanks (600 × 300 × 330 mm) were constructed from clear acrylic (poly methyl methacrylate) with a working volume of 46 L. Temperature of the FSW was adjusted to 27 °C prior to entering the tank and each tank sat inside a water bath for further temperature control. A submersible recirculating pump within each tank provided the water flow required to maintain optimal coral health and a homogenous solution with adequate aeration. Lighting used in the acclimation and experimental phases simulated the photoperiod and light intensity of field conditions with a 2-h sunrise, 8-h daylight (100–150 μmol/m^2/s photosynthetically active radiation), 2-h sunset, and 12-h night and Daily Light integral (DLi) of 5.8 mol/m^2/d.

Water quality measurements (temperature, pH, salinity, specific electrical conductivity (spEC), dissolved oxygen (DO)) were made in the tank solutions using a YSI-ProDSS multi-probe and portable pH probe (PHC101 probe, HQ40d Hach). Ammonia was measured using a colourimetric test kit (API®). Dissolved organic carbon (DOC) was analysed in filtered (<0.45 μm) samples by high temperature combustion of the sample in the presence of a catalyst with the resulting carbon dioxide quantified using an infrared detector (APHA (2017) 5310B, LOD = 1 mg/L).

Truly dissolved, i.e., ultrafiltered (<3 kDa Macrosep, Pall centrifugal device), dissolved i.e., filtered (<0.45 μm, Minisart Sartorius) and total i.e., unfiltered metals were operationally defined by filter pore size and analysed as described below.

FSW at the SeaSim was used as the diluent and had the following dissolved background metal(loid)s concentrations: 4.4 μg Al/L; 1.4 μg As/L; <0.01 μg Cd/L; <0.01 μg Co/L; 0.13 μg Cr/L; 0.36 μg Cu/L; 0.42 μg Fe/L; 0.47 μg Mn/L; 11 μg Mo/L; 0.29 μg Ni/L; <0.02 μg Pb/L; 0.26 μg Se/L; 2.0 μg V/L; <0.1 μg Zn/L.

The manganese dosing parent stocks (5 g Mn/L and 10 g Mn/L) as $MnCl_2.4H_2O$ (Sigma Aldrich analytical grade ACS, 99.1 % purity) were prepared in bulk with unacidified deionized water (18 MΩ/cm Milli-Q, Millipore) to prevent precipitation of manganese in seawater. The stock concentrations were below the manganese chloride solubility limit of 770 g/L (Aylward and Findlay, 2008) and the dosing volumes were small enough to not notably affect the salinity of the dosed seawater. The working stocks in the 14-d experiment were dilutions of the parent stocks in FSW.

2.2. Collection and acclimation of Acropora millepora

Four genotypes from partial colonies of adult corals (*Acropora millepora*) were collected from Davies Reef (−18.83S, 147.63E, GBRMPA permit number G21/38062.1) in the central Great Barrier Reef. On return to the SeaSim facility, colonies were held in a short-term holding system prior to fragmenting, where the water temperature was matched to the sea-surface temperature (SST) at the location and time of collection (29 °C). Colonies were fragmented (7–9 cm fingers) and mounted onto both aragonite and ceramic coloured (blue, green, black and white for genotype identification) plugs using adhesive (Gorilla Super Glue Gel). The fragments were arranged on polyvinylchloride (PVC) racks, according to genotype and moved to a recirculating system with biological filtration and temperature control for acclimation to temperature and artificial lighting. Acclimation tank flowrate was 7 L/min of FSW to help maintain water quality. Water temperature was incrementally reduced to the experimental temperature of 27 °C over 3 weeks. Coral nutrition was provided every afternoon in the form of microalgae (*Chaetocerus muelleri*, *Nannochloropsis* sp., *Proteomonas sulcata*, *Dunaliella* sp., *Tisochrysis lutea* (formerly known as Tahitian *Isochrysis*) fed at densities of 2000 cells/mL) and zooplankton (*Brachionis* sp. (Rotifers), fed at 1 rotifer/2 mL, and *Artemia salina* fed at 1 nauplii/2 mL). Total ammonia was maintained at <0.1 mg NH_3-N/L during the acclimation period. At the time of field coral collection, many coral species were beginning to exhibit signs of bleaching due to temperature stress. However, *A. millepora* were not bleached and following four weeks of acclimation, the coral fragments showed new tissue growth at the basal connection to plugs and on any lesions incurred during the fragmentation process. Following acclimation, the corals had a survival rate > 95 % and were considered to be in suitable physiological condition for use in the experiments.

Table 1
Experimental conditions for acute and chronic exposures of adult coral *Acropora millepora* to dissolved (<0.45 μm filtered) manganese.

Parameters	2-d acute	14-d chronic
Temperature (°C)	27	27
pH	8.1	8.1
Dissolved oxygen (% saturation)	>90	>90
Specific conductivity (mS/cm at 25 °C)	54	54
Salinity (PSU)	35	35
Total ammonia (mg N/L)	<0.1	<0.1
Photoperiod (h)	2-h sunrise, 8-h daylight, 2-h sunset, and 12-h night	2-h sunrise, 8-h daylight, 2-h sunset, and 12-h night
Light intensity (μmol photons/m^2/s photosynthetically active radiation (PAR))	100–150 μmol/m^2/s PAR, Daily Light integral (DLi) of 5.8 mol/m^2/d	100–150 μmol/m^2/s PAR, Daily Light integral (DLi) of 5.8 mol/m^2/d
Exposure system	Static 50 % renewal prior to dosing on Day 0 and Day 1	Flow-through (24 tank volume turn-overs/replicate/d)
Chamber (L)	50	50
Solution volume (L)	46	46
Nominal concentrations (*replicate tanks per concentration*) (μg Mn/L)	0 (*3*), 1000 (*3*), 2500 (*3*), 5000 (*3*), 10,000 (*3*), 20,000 (*3*)	0 (*4*), 10 (*2*), 20 (*2*), 40 (*2*), 60 (*2*), 80 (*2*), 100 (*2*), 150 (*2*), 200 (*2*), 300 (*2*), 400 (*2*), 500 (*2*), 1000 (*1*)
Diluent water type	Natural seawater (<1 μm filtered)	Natural seawater (<1 μm filtered)
Source of coral	Davies Reef, Great Barrier Reef	Davies Reef, Great Barrier Reef
Acclimation time	4 weeks in holding tanks and 1 d in treatment tanks prior to dosing	4 weeks in holding tanks and 2 d in treatment tanks prior to dosing
Life stage of coral	Adult (7–9 cm fragment length)	Adult (7–9 cm fragment length)
Number of coral fragments per replicate	4 (all were used for health assessment)	9 (6 were used for health assessment)
Food	No feeding during experiment	No feeding during experiment
Biological endpoint	Coral health % relative to control based on tissue sloughing	Coral health % relative to control based on tissue sloughing
Acceptability criterion	≥90 % coral health and survival in controls	≥90 % coral health and survival in controls

2.3. 2-d acute manganese experiment

A single experiment was performed with a static-renewal exposure system using FSW controls and five nominal manganese treatments (1000, 2500, 5000, 10,000, 20,000 μg Mn/L), with three replicate tanks per treatment (Table 1).

On Day −1, four healthy, acclimated, adult corals representing the four genotypes were randomly selected from the holding tanks, uniquely labelled (with sequential numbering on waterproof labels attached to the plug with adhesive) and allocated to each treatment tank containing FSW to acclimate for a further 24 h. On Days 0 and 1, 23 L of FSW in each tank was replaced (i.e., 50 % renewal) by siphon without disturbing the coral, and aliquots of the manganese dosing stocks were added. Coral health was assessed as described below using in situ (i.e., in water) photos of the coral taken on Day 0 prior to exposure to manganese and 4 h post-exposure, Days 1 and 2. Corals were not fed during the 2-d experiment.

Filtered metals were measured in each tank on Days 0 and 2 and before and after the 50 % renewal on Day 1. Ultrafiltered and unfiltered metals and DOC were measured in selected treatments (0, 2500, 5000 and 10,000 μg Mn/L) on Days 0 and 2. Water temperature, pH, spEC, salinity and DO were measured in each tank on Days 0 and 2 and before and after the water renewal on Day 1. At the same time points, total ammonia was measured in a composite sample of the replicate tanks for each treatment.

2.4. 14-d chronic manganese experiment

A single experiment was performed with a flow-through exposure system using FSW controls (four replicate tanks) and 12 manganese treatments (two replicate tanks per treatment at 10–500 μg Mn/L and one tank at 1000 μg Mn/L) (Table 1, Fig. S1). Manganese working stock solutions (25 L in 46 L clear acrylic tanks, at 2000–200,000 μg Mn/L) were topped up daily by adding aliquots of manganese parent stock (10 g Mn/L) to 10 L FSW batches that were then transferred into the working stock tank. Each manganese working stock was diluted 200-fold by the FSW in the dosing lines to reach the final required manganese concentration in each replicate tank. FSW flowing at 1.6 L/min was blended with manganese working stocks (2–200 mg Mn/L) flowing at 8 mL/min using a calibrated Ismatec IPC peristaltic pump (ISM932D) and Tygon® tubing (Idex Isamatec, 1.52 mm internal diameter, yellow/blue) to give a final flow rate of 0.8 L/min/replicate or 24 tank volume turn-overs/replicate/d. Each manganese supply line (John Guest low density polyethylene) was split to the required number of replicate tanks per treatment that were randomly located within the controlled environment room. Treatment solutions entered at the top of each tank and exited at the bottom of each tank to a waste line where it was treated with activated carbon to remove manganese prior to release.

On Day −2, nine healthy adult coral representing triplicates of three genotypes (green, blue and black) were randomly selected from the acclimation tanks, uniquely labelled, and allocated to each treatment replicate tank containing FSW-only for a further 48-h acclimation prior to dosing with manganese. Six of the coral fragments were used for the coral health assessment and three coral fragments were used for a separate study not reported on here. The manganese exposure commenced on Day 0 by starting the peristaltic dosing pump in parallel with adding aliquots of manganese stock directly to each treatment tank so that within 45 min of starting the pump, all treatment tanks reached the nominal manganese concentration. Coral health was assessed as described below using ex situ (i.e., out of water) photos taken on Day −2, and in situ photos taken on Days 0, 7 and 14. Visual observations were also recorded daily. Over the 14 d, filamentous algae grew on the tank walls of all treatments and were removed from the front tank wall using a plastic scraper to enable an unobstructed view of the coral fragments for the in situ health assessments. Corals were not fed during the 14-d experiment.

Filtered and unfiltered metals were measured in each tank on Days 0 and 14. On those same days, ultrafiltered metals and DOC were measured in all tanks of selected treatments (0, 10 and 500 μg Mn/L). Additional filtered metals were measured in each tank on Day 2. Water temperature, pH, spEC, salinity and DO were measured in each tank on Day 0 and then in one replicate tank for each treatment on Days 1, 3, 7, 9, 10, 12 and 14. Total ammonia was measured in a composite sample of the replicate tanks for each treatment on Days 0, 4, 7, 10, 12 and 14.

Table 2
Acropora millepora health assessment and scoring based on the tissue sloughing biological endpoint (see Table S2 for more detail).

Criterion	Description	Score
Healthy (stage 0 slough)	• Tissue and *Symbiodinium* spp. are clearly intact giving a 'pattern' (often striped) to the tissue • Polyp tips have a 'fleshy' look • No sign of bleaching • Tentacles are usually obvious	4
Impaired health (stage 0 slough)	• Presence of mesenterial filaments, mucus • Tentacle retraction (may be partial) • Note: tentacle retraction is based on a comparison to Time 0 • Note: score of 3 was not based on tentacle retraction alone, but tentacle retraction sometimes accompanied the presence of mesenterial filaments	3
Stage 1 slough	• Some polyp tips are showing sloughing where tissue has a 'ragged' appearance rather than 'fleshy' when in situ • Clearly some healthy poly tips visible • *Symbiodinium* spp. seem intact and 'pattern' evident, but some clumps may be visible • Tentacles may be retracted • Sub-lethal	3
Stage 2 slough	• Throughout the colony most polyp tips are sloughing, tissue has a 'ragged' appearance rather than 'fleshy' at the tip when in situ • *Symbiodinium* spp. seem intact and 'pattern' evident, clumps are visible throughout • Little to no tentacles are visible • Likely lethal	2
Stage 3 slough	• Advance sloughing, with some patches of tissue disintegrated • Clear evidence that tissue is compromised • *Symbiodinium* spp. clumps visible throughout, and 'pattern' is compromised in places • Skeleton on polyp tips is exposed • Recovery not possible	1
Dead	• Obvious tissue disintegration • White skeleton is clearly visible, • *Symbiodinium* spp. pattern in tissue is not evident • Recovery not possible	0

2.5. Assessment of A. millepora health

The health of four and six adult coral fragments in respective acute and chronic experiment tanks, was assessed using visual observations and photographs of coral fragments that were in situ (for the acute and chronic experiments) and ex situ (for the chronic experiment on Day −2 only). In situ visual observations and photography were made on coral fragments positioned on a rotating platform made of PVC that enabled each coral fragment to be moved to the front window of the tank without manual handling, and provided the same coral perspective at each time point (Fig. S2). Ex situ photos of coral were taken with the coral on a platform in a fixed position and distance from the camera (Fig. S3). The same camera (either an Olympus Tough TG-5 or TG6) was used to photograph the same coral fragment throughout the experiment to maintain a consistent quality of images over time. A minimum of two photos per coral fragment were taken with the camera on the Microscope setting, varying magnification and no flash.

The method of coral health assessment was modified from that developed for another coral species (*A. muricata*) by Binet et al. (2023b) and involved scoring the health of each coral from zero to four based on visible stages of tissue sloughing (Tables 2 and S2 for more detail).

The same scoring system was used in both the acute and chronic experiments and the biological endpoint was calculated by dividing the score for each coral fragment by four (the optimum score) to give a percentage of coral health based on tissue sloughing. This was then normalised for the control and averaged across the corals in the tank resulting in the final biological endpoint which was the average percentage of coral health (based on tissue sloughing) relative to the control for each replicate tank. In addition to the photos, visual observations and notes were made daily to record whether: mucus was produced; tentacles were retracted or swollen; mesenterial filaments (MFs), as defined by Woodley et al. (2016), were active; polyp tissue was ragged; and loss of tissue had occurred.

Assessment of coral bleaching in the chronic experiment was based on daily visual observations for initial signs of bleaching such as a decrease in coral fragment colour intensity in the manganese treatments relative to the controls. If these signs were observed during the experiment, coral bleaching would be quantified at 14 d by image analysis software that measured coral colour intensity (average pixel values on the greyscale) from ex situ photos according to the methods described by Binet et al. (2023b) and Gillmore et al. (2020).

The criteria for acceptable coral health and survival in the controls for both the acute and chronic experiments were defined as ≥90 %. A parallel reference toxicant experiment was not conducted for the acute and chronic experiments because a historical database does not exist for this species.

2.6. Metals analyses

Ultrafiltered, filtered and unfiltered water samples were collected directly from the treatment tank using acid washed syringes (20 mL, Terumo, 10 % nitric acid washed and deionized water rinsed). For ultrafiltered and filtered samples, 30 mL and 10 mL of sample, respectively, was passed through a 0.45 μm syringe filter to waste to precondition the filter prior to collecting the actual filtered sample in acid washed polycarbonate tubes (10 or 30 mL). Ultrafiltered samples (30 mL) were filtered further in a centrifugal device (Macrosep, Pall) that had been pre-conditioned by rinsing the device with deionized water without the filtration cartridge followed by sequential rinses of the device with the filtration cartridge by centrifuging (Beckman Coulter Allegra X-15R set to 27 °C) 15 mL of deionized water (10 min at 1230 ×*g*), rinsing with 15 mL of 10 % nitric acid (TracePur, Merck; 10 min at 1230 ×*g*), rinsing with 15 mL of deionized water (10 min at 1230 ×*g*), and rinsing with 15 mL of sample (10 min at 1230 ×*g*). The final sample was processed in the pre-conditioned device by centrifuging 15 mL sample for 35 min at 1230 ×*g* and transferring 10 mL into an acid washed polycarbonate tube. Each sample was processed with a new centrifugal device to eliminate cross-contamination. Method blank samples were collected after the first and second deionized water rinses to check for possible contamination. All metal samples were preserved with 0.2 % vol:vol concentrated nitric acid (TracePur, Merck).

All control and treatment water samples were analysed for manganese and selected samples were analysed for multiple metal(loid)s (limit of detection (LOD): 0.4–1 μg Al/L; 0.1–1 μg As/L; 0.01–0.1 μg Cd/L; 0.01–1 μg Co/L; 0.01–0.2 μg Cu/L; 0.01–0.5 μg Cr/L; 0.3–1 μg Fe/L; 0.02–1.0 μg Mn/L; 0.02–0.5 μg Ni/L; 0.02–0.5 μg Pb/L; and 0.1–1 μg Zn/L) using inductively coupled plasma atomic emission spectrometry (ICP-AES, Agilent 5110 SVDV) and ICP-MS (Perkin Elmer NexION 300D and 350D and Agilent 8800). Matrix-matched standards were used as well as drift calibration standards (<20 % drift), duplicate samples (relative percent difference (RPD) = 1–4 % for Mn; acceptable RPD is <30 %), spike recoveries (98 % Mn recovery) and five certified reference materials (recovery = 83–106 % Mn).

2.7. Data analyses

The concentration-response curve was a function of the measured time-averaged dissolved (<0.45 μm filtered) manganese concentration versus the average coral health percentage (relative to the control) for each replicate tank. From this, the effect concentration at which the coral health was 10, 20 or 50 % (i.e., EC10, EC20, EC50, respectively) of the control in the acute and chronic experiments was derived using the R package drc (R Core Team, 2022; Ritz et al., 2015). Selection of the best fitting model was based on the lowest Akaike Information Criterion (Pinheiro and Bates, 2000), the lowest standard error of the fit and visual fit assessment. The 95 % confidence limits associated with the effect concentrations were estimated using the delta method (Ritz et al., 2015). The NOEC was determined in the acute experiment using a two-sample *t*-test between the control and the 2500 μg Mn/L treatment following the testing of assumptions of normality and equality of variance using NCSS (7.1.21, Hintze (2007)). Statistically significant differences in the sensitivity between the four genotypes of *A. millepora* used in the acute experiment, were tested using a full model, two factor (manganese concentration and genotype) analysis of variance (ANOVA) on the log(x + 1) transformed 48-h average tissue sloughing score for each coral fragment using NCSS. Assumptions of normality and equality of variance were tested for each individual factor prior to performing the two factor ANOVA.

In addition to the toxicity values from the acute and chronic experiments with *A. millepora*, the literature was reviewed for acute and chronic manganese toxicity data according to the guidance from Warne et al. (2018). Toxicity values were assessed and scored using a range of criteria that included the use of measured manganese exposure concentrations, standard methods and experimental design and only those that scored ≥50 % were categorized as 'acceptable' and included in the toxicity database. The 'acceptable' quality data were screened further to remove acute values given that there were sufficient preferred chronic values and to remove those values that were based on nominal manganese concentrations. The exception to this was the acute adult coral tissue sloughing toxicity values one of which was based on nominal manganese concentration. These acute values for coral were retained because of the high sensitivity compared to endpoints for most other marine biota and the ecological and economic importance of coral and the reef ecosystem they support. Acute EC50 values for adult coral tissue sloughing were converted to estimated chronic values by dividing the acute EC50 values by the ACR of 2.3 derived from the acute EC50 divided by the chronic NOEC for *A. millepora* in this study. The remaining chronic toxicity data were passed through a selection process to produce one toxicity value per species. A preference of selection was given to toxicity values in order of the chronic no effect concentration (NEC) > EC10 > NOEC values for use in the SSD.

Two software packages, Burrlioz 2.0 and the ssdtools Shiny App (Dalgarno, 2018) referred to hereon as shinyssdtools 0.1.1 were used to

Table 3
Average physico-chemical measurements taken from control and treatment tanks during the acute and chronic exposures of *Acropora millepora* to dissolved (<0.45 μm filtered) manganese (standard deviation shown in parentheses).

	Temperature	pH	DO[a]	spEC[b]	Salinity	DOC[c]	Total ammonia
	°C		% saturation	mS/cm	psu	mg/L	mg N/L
Acute (2 d)							
Control	27 (0.3)	8.1 (0.03)	100 (0.4)	54 (0.1)	36 (0.1)	1.3 (0.5)	<0.1
Mn	27 (0.3)	8.1 (0.02)	100 (0.6)	54 (0.2)	35 (0.2)	1.0 (0)	<0.1
Chronic (14 d)							
Control	27 (0.1)	8.1 (0.06)	100 (3)	53 (0.1)	35 (0.1)	1.3 (0.5)	<0.1
Mn	27 (0.2)	8.1 (0.06)	100 (3)	53 (0.1)	35 (0.1)	1.3 (1)	<0.1
All	**27 (0.3)**	**8.1 (0.06)**	**100 (3)**	**54 (0.2)**	**35 (0.2)**	**1.2 (0.7)**	**<0.1**

Values in bold are the average and standard deviation for all measurements across treatments and experiments (n = 40–190).
[a] Dissolved oxygen.
[b] Specific electrical conductivity at 25 °C.
[c] Dissolved organic carbon limit of detection was 1.0 mg/L.

generate SSDs from the final set of toxicity values assessed from the literature and combined with the coral data from this study. The software packages differ in the types of models they apply to the data and whether a single model or an average of models is applied (Fox et al., 2022). Currently, SSDs derived using Burrlioz is the recommended approach for deriving water quality GVs in Australia and New Zealand and applies a single model to fit the data. In contrast, shinyssdtools applies up to six models as the default option, and determines the average model fit. Water quality GVs that provide protection to 80, 90, 95 and 99 % of marine biota were derived directly from the SSD without additional assessment factors being applied (Fox and Batley, 2022). The GVs were assigned a level of reliability based on the number of chronic toxicity values used, the size of the dataset and an assessment of the curve fit to the data in the SSD (Warne et al., 2018). The Burrlioz software was ultimately used to derive the final GVs however, the GVs derived using shinyssdtools may be useful if it is adopted in the future (Fox et al., 2022).

3. Results/discussion

3.1. Physico-chemistry

The physico-chemistry of the acute static-renewal and chronic flow-through systems remained stable throughout the duration of the experiments and showed no marked differences between controls and manganese treatments (Table 3). These conditions ensured optimum health of the corals in the controls.

3.2. Manganese speciation and dosing

Measurements of the unfiltered and filtered concentrations of manganese in the acute and chronic experiments confirmed that in both experiments, there was no marked loss of manganese by precipitation or adsorption to surfaces and that all manganese was present in the truly dissolved form (<3 kDa) without significant formation of colloidal

Table 4
Toxicity of ultrafiltered (<3 kDa), filtered (<0.45 μm) and unfiltered manganese to adult coral (*Acropora millepora*) health in acute and chronic experiments.

Nominal Mn	Ultrafiltered Mn	Filtered Mn	Unfiltered Mn	Coral health	n[a]
(μg/L)	(μg/L)	(μg/L)	(μg/L)	(%)	
Acute (2 d)					
0	<1 (0.0)	<1 (0.0)	<1 (0.0)	100 (0.0)	2 or 3
1000	–	910 (16)	–	100 (0.0)	2 or 3
2500	2300 (64)	2400 (76)	2300 (13)	54 (18)	2 or 3
5000	5000 (15)	5100 (290)	5000 (250)	15 (3.6)	2 or 3
10,000	9900 (100)	9800 (110)	10,000 (200)	13 (6.3)	2 or 3
20,000	–	23,000 (130)	–	4.2 (7.2)	2 or 3
Chronic (14 d)					
0	<1 (0.0)	<1 (0.0)	<1 (0.0)	100 (0.0)	2 or 4
10	10 (0.24)	11 (0.93)	11 (0.10)	100 (0.0)	2
20	–	22 (0.50)	21 (1.2)	100 (0.0)	2
40	–	40 (0.59)	39 (5.8)	100 (0.0)	2
60	–	65 (1.6)	61 (3.7)	100 (0.0)	2
80	–	89 (0.34)	83 (3.0)	100 (0.0)	2
100	110 (0.022)	110 (5.3)	110 (4.1)	100 (0.0)	2
150	–	170 (2.4)	170 (13)	98 (2.9)	2
200	–	200 (2.9)	200 (13)	100 (0.0)	2
300	–	330 (6.6)	330 (1.4)	100 (0.0)	2
400	–	440 (11)	410 (15)	100 (0.0)	2
500	510 (4.0)	570 (0.56)	500 (4.0)	100 (0.0)	2
1000	–	1100	970	100	1

Time-averaged concentrations and average coral health (and standard deviations) are reported to two significant figures. [a]Time averaged concentrations were calculated in two replicates for ultrafiltered and unfiltered manganese and three replicates for filtered manganese in the acute experiment. Two replicates were used for ultrafiltered and unfiltered manganese and four replicates were used for filtered manganese in the control of the chronic experiment. Acute toxicity was measured in all three replicates for each treatment, and chronic toxicity was measured in four control replicates, two replicates for 10–500 μg Mn/L and one replicate for 1000 μg Mn/L.

(subtraction of the ultrafiltered fraction from the filtered fraction) manganese (Table 4). Filtered manganese concentrations were 94–104 % and 92–116 % of the unfiltered manganese in respective acute and chronic experiments without any trends related to increasing manganese treatment concentration or exposure time as may be expected if solubility was an issue. Ultrafiltered manganese concentrations were 96–105 % and 94–106 % of unfiltered manganese concentrations in the respective acute and chronic experiments which suggests that the manganese was present in the most bioavailable truly dissolved Mn(II) oxidation state rather than the intermediary Mn(III) or fully oxidised Mn (IV) states that form colloidal or solid phases and are less bioavailable.

The acute static-renewal and chronic flow-through systems performed with a high degree of dosing accuracy, as demonstrated by filtered manganese time-averaged concentrations being 90–114 % and 99–117 % of the respective acute and chronic target nominal concentrations. There was −16 to 15 % and −24 to 11 % change in filtered manganese concentration between time interval measurements in the respective acute and chronic experiments which is within the acceptable analytical precision limit of <30 % RPD. An unplanned power outage in the chronic flow-through experiment resulted in the manganese dosing pump being off for 9 h on the tenth day of the 14-d chronic experiment which equated to 3 % of the total experiment duration. During this time the dissolved manganese dropped to background concentrations in all dosing treatments as FSW continued to flow through the system. The target nominal dissolved manganese concentrations were resumed in all tanks within 1 h of re-starting the dosing pump and the effect of this small dosing interruption on the corals' final chronic response was assumed to be negligible given that the response of coral in the 1000 μg Mn/L treatment of the separate acute and chronic experiments was the same. Dissolved metals in the controls and treatments (other than manganese) remained generally close to background FSW concentrations with some low level, random inconsistencies in the acute (maximum values: 23 μg Al/L; 2.8 μg As/L; 2.9 μg Cr/L; 3.6 μg Cu/L; 19 μg Fe/L; 10 μg Ni/L; 2.3 μg Zn/L) and chronic experiments (maximum values: 12 μg Al/L; 2.5 μg As/L; 0.4 μg Cu/L; 1.6 μg Fe/L; 2.8 μg Mn/L (control on one occasion); 2.1 μg Zn/L) that may have been due to sample handling contamination. Manganese concentration was below the LOD in the ultrafiltration device method blanks.

3.3. Acute toxicity of dissolved manganese to A. millepora

Coral health and survival was 100 % in the controls and therefore met the experiment acceptability criterion (Tables 1 and 4). The 2-d acute experiment resulted in NOEC, EC10, EC20 and EC50 values of 906, 1300, 1590, and 2560 μg Mn/L, respectively for adult *A. millepora* tissue sloughing (Tables 4 and 5, Figs. 1 and 2a). Acute toxicity of manganese to the corals was observed within the first 4 h with 42 ± 14 % (mean ± standard deviation, $n = 3$) and 100 % of the corals in the respective 2500 μg Mn/L and ≥5000 μg Mn/L treatments releasing excessive amounts of mucus. In addition, 100 % of corals in the ≥2500 μg Mn/L treatments had retracted tentacles over most or all of the fragment. After 1 d, mucus production was no longer visible in the corals exposed to the 2500 μg Mn/L treatments but tentacles were still retracted and at Stage 1 of tissue sloughing in 67 ± 14 % of corals. Corals in the ≥5000 μg Mn/L treatments continued to release mucus which was sometimes black in colour, had retracted tentacles and were at the more advanced Stage 2 of tissue sloughing (Tables 2 and S2). By 2 d, no further mucus was being produced by any corals, but corals in the 2500 μg Mn/L treatment still had retracted tentacles and were at Stage 2 or 3 of tissue sloughing, while those corals in the ≥5000 μg Mn/L treatments

Table 5
Acute and chronic toxicity values (μg Mn/L) for coral species and *Symbiodinium* spp. exposed to dissolved (<0.45 μm filtered) manganese.

Species	Life stage	Exposure duration (d)	Type	Biological endpoint	NOEC (μg/L)	EC10 (μg/L)	EC20 (μg/L)	EC50 (μg/L)	Reference
Acropora millepora	Adult	14	Chronic	Tissue sloughing	≥1090	–	–	–	This study
	Adult	2	Acute	Tissue sloughing	906	1300 (910–1690)	1590 (1220–1970)	2560 (2220–2900)	This study
Acropora muricata	Adult	2	Acute	Tissue sloughing	–	227 (19–436)	–	728 (399–1100)	Binet et al. (2023b)
	Adult	2	Acute	Tissue sloughing	–	281 (57–505)	–	933 (662–1200)	Binet et al. (2023b)
Acropora spathulata	Adult	2	Acute	Tissue sloughing	–	50[a]	–	700[a]	Summer et al. (2019)
	Adult	2	Acute	Mortality	–	1800[a]	–	2700[a]	Summer et al. (2019)
	Larva	3	Acute	Mortality	–	–	–	7000[b]	Summer et al. (2019)
	Larva	1	Acute	Mortality	17,000	4000[b] (0–9000)	–	28,000 (24,000–23,000)	Summer et al. (2019)
	Gamete	5.5 h	Chronic	Fertilisation[c]	72,000	15,000 (0–38,000)	–	237,000 (199,000–304,000)	Summer et al. (2019)
Platygyra daedalea	Gamete	5.5 h	Chronic	Fertilisation[c]	54,000	43,000 (33,000–58,000)	–	164,000 (125,000–249,000)	Summer et al. (2019)
Stylophora pistillata	Adult	2	Acute	Tissue sloughing	510	–	–	860 (700–1000)	Stauber et al. (2002)
	Adult	2	Acute	Mortality	1100	–	–	1500	Stauber et al. (2002)
Dinoflagellates isolated from coral *S. pistillata*	–	2	Acute	Quantum yield	1000	–	–	>1000	Stauber et al. (2002)
	–	2	Acute	Dinoflagellate density	1000	–	–	>1000	Stauber et al. (2002)
Dinoflagellates isolated from coral *Heliofungia actiniformis*	–	6 h	Acute	Quantum yield	>200,000	–	–	–	Stauber et al. (2002)

Values are reported to no more than three significant figures. 95 % confidence limits are shown in parentheses where available.
[a] Toxicity value is based on nominal manganese and was part of a pilot trial with low replication, whereas all other toxicity values are based on measured dissolved (<0.45 μm filter) manganese.
[b] Toxicity estimate is extrapolated below the lowest test concentration.
[c] Results for fertilisation could not be replicated and the use of the NOEC was suggested as being more appropriate than the EC10 or EC50 (Summer et al., 2019).

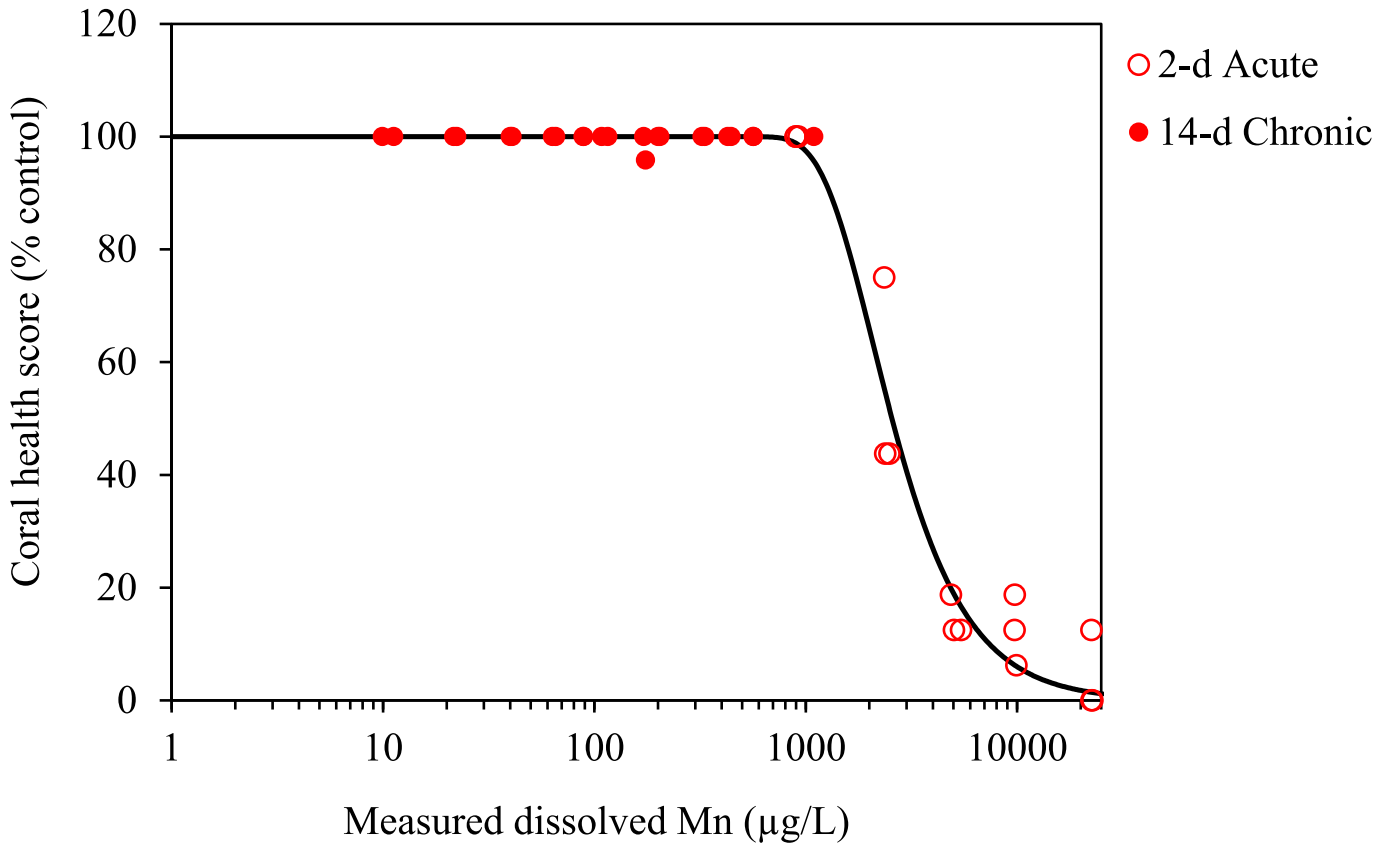


Fig. 1. Concentration-response relationship between acute (2 d) and chronic (14 d) response of adult *Acropora millepora* corals, and measured dissolved (<0.45 µm filtered) manganese. Coral health is determined by scoring stages of tissue sloughing. Each point is the average percent coral health score for the corals in a treatment tank. The coral in the control tanks each had an average score of 100 % and could not be shown on the log scale.

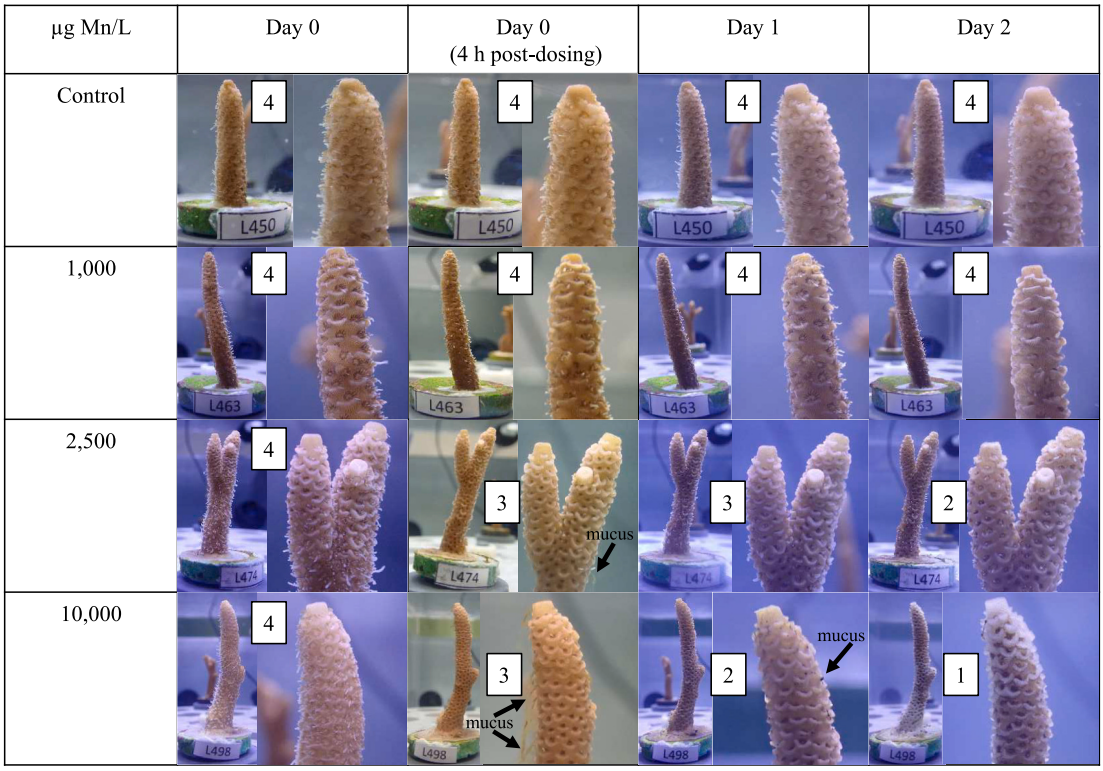
were dead with the majority of tissue lost. There were no visible signs (i.e., decline of tissue colour intensity) of bleaching of tissue prior to tissue sloughing which is consistent with other coral species exposed to dissolved manganese (Binet et al., 2023b; Stauber et al., 2002; Summer et al., 2019). Corals in the control and 1000 µg Mn/L treatments showed no excessive mucus production, no permanent retraction of tentacles (although temporary tentacle retraction was observed), no bleaching (based on visual observations) and no tissue sloughing over the 2-d experiment.

Mucus is a polysaccharide protein-lipid complex secreted by corals at their surface but is also composed of the dissolved organic carbon produced by the *Symbiodinium* spp. during photosynthesis. Mucus has a diverse array of functions in corals such as a protective barrier to external stressors, defence against other corals and heterotrophic feeding (Brown and Bythell, 2005). External stressors include ultraviolet radiation, desiccation, smothering by sediment, increased water temperature, decreased salinity and contaminant exposure (Brown and Bythell, 2005). Mucus has been shown to accumulate higher contaminant concentrations than coral tissue and may contribute to regulating contaminant exposure to corals in a similar way to *Symbiodinium* spp. (Kang et al., 2022; Han et al., 2020; Reichelt-Brushett and McOrist, 2003; Zhang et al., 2019). Excessive mucus production also occurred within 24 h of the acute manganese experiment with *A. muricata*, beginning at much lower dissolved concentrations of ≥250 µg Mn/L when compared to *A. millepora* (Binet et al., 2023b). Therefore, while mucus production is highly variable between coral species and there are multiple functions of mucus production, *A. millepora* produced excess mucus as a response to acute dissolved manganese exposure and this could be an additional coral endpoint to analyse and quantify in future research.

Coral tentacle retraction may have been a direct neurotoxicity effect of manganese and also occurred in adult *A. muricata* at dissolved concentrations ≥250 µg Mn/L after 24 h, which is again at a much lower concentration when compared to *A. millepora* (Binet et al., 2023b). Another closely related cnidarian, the anemone (*Exaiptasia pallida*), also displayed tentacle retraction when exposed to dissolved manganese concentrations of 460 to 87,000 µg Mn/L for 12 d followed by partial recovery after 6 d in non-contaminated seawater (Iyagbaye et al., 2022). However, Summer et al. (2019) did not observe any effects on *E. pallida* tentacle retraction during 12-d exposure to 4600–54,000 µg Mn/L which is at least 100 fold higher in concentration than where effects on tentacles were observed by Iyagbaye et al. (2022) and demonstrated that tentacle retraction was a highly variable response in *E. pallida*. In contrast, coral tentacle retraction was a reliable biological endpoint when incorporated with other tissue sloughing indicators in the coral health assessment used in this study (Tables 2 and S2).

The fact that loss of tissue was not preceded by loss of *Symbiodinium* spp. i.e., bleaching, suggests that the *Symbiodinium* spp. have a higher tolerance to dissolved manganese than the host. No adverse effects (based on photosynthetic yield) on *Symbiodinium* spp. extracted from coral (*S. pistillata*) occurred at 1000 µg Mn/L (Stauber et al., 2002). Binet et al. (2023b) observed an increase in colour intensity of coral tissue in *A. muricata* exposed to manganese which is the opposite to bleaching and suggestive of an increased density or pigment concentration of *Symbiodinium* spp. but this also occurred in the control corals. Iyagbaye et al. (2022) observed no effects on *Symbiodinium* spp. density in the anemone, *E. pallida*, at concentrations up to 100,000 µg Mn/L over 24 d. *Symbiodinium* spp. have been shown to accumulate and store metals including manganese at higher concentrations than coral tissue suggesting that mechanisms to detoxify and store manganese exist in

(a) Acute experiment



(b) Chronic experiment

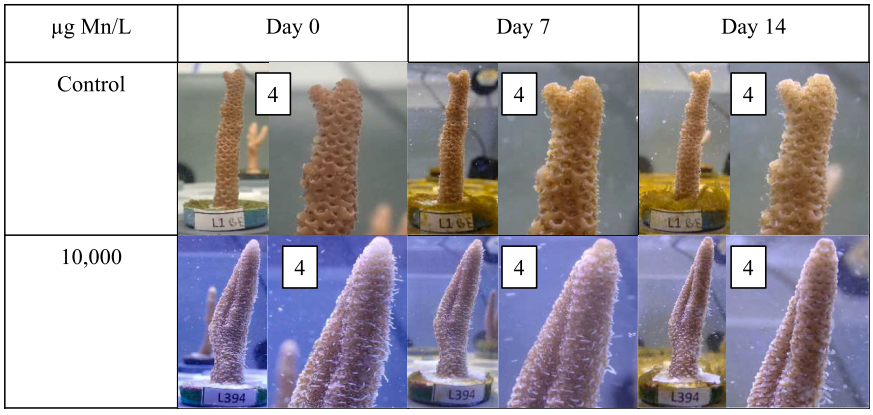


Fig. 2. Examples of in situ images showing effects on *Acropora millepora* observed over time when adults were exposed to dissolved manganese in the (a) 2-d acute, and (b) 14-d chronic experiments. The whole fragment and magnification of the same fragment are shown for each concentration and time point. Numbers 1–4 are the coral health assessment scores for the individual fragment. Mucus is shown. Please see Tables 2 and S2 for interpretation of the score.

Symbiodinium spp. as is the case for other microalgae and diatoms (Metian et al., 2015; Reichelt-Brushett and McOrist, 2003). These data suggest that the role of manganese as an essential element for photosynthesis and for anti-oxidant enzymes means that the threshold for toxicity is higher for *Symbiodinium* spp. than cnidarians in the species studied thus far and bleaching is not expected to be an early indicator of manganese toxicity to corals.

There were statistically significant differences in manganese sensitivity between genotypes of *A. millepora* at concentrations ≥2500 µg Mn/L and no differences between genotypes in the control and 1000 µg Mn/L treatments (Table S4). The assumptions of normality and equal variance were met for the genotype factor but only the assumption of normality was met for the manganese concentration factor despite the log(x + 1) transformation of the coral health score, so the results of this analysis should be interpreted with caution. The 'white' and 'blue' coded genotypes of *A. millepora* were consistently the most and least tolerant, respectively, of manganese at concentrations ≥5000 µg Mn/L. This suggests that there are differences in the toxic threshold between genotypes with increasing manganese concentration as measured by tissue sloughing. Such differences between genotypes are likely to be detected at lower manganese concentrations when measuring subcellular responses rather than tissue sloughing. The possibility of differences in metal sensitivity between coral genotypes needs to be factored into the experimental design of future research as was done in this experiment,

to account for natural variation within a species.

Adult *A. millepora* corals were 3–4 fold more tolerant to manganese than the three other coral species (*A. muricata*, *A. spathulata*, *S. pistillata*) based on the acute 2-d EC50 values for tissue sloughing (Table 5). This was an unexpected result given that the acute EC50 values for the three other coral species are so similar (Table 5). When the toxicity of a different metal (copper), for the only available endpoint of 4–5 h fertilisation success, is compared between *A. millepora* (EC50 of 17 μg Cu/L) and other coral species (EC50 values of 45–75 μg Cu/L), there is no trend of greater tolerance by *A. millepora* (Gissi et al., 2017; Negri and Heyward, 2001; Puisay et al., 2015; Victor and Richmond, 2005). Therefore, the slightly higher tolerance of *A. millepora* is specific to manganese and a better understanding of the mechanism of manganese toxicity to adult corals (Binet et al., 2023b) may help to explain the interspecies differences in sensitivity.

3.4. Chronic toxicity of dissolved manganese to A. millepora

Control coral health and survival in the 14-d experiment was 100 % and therefore met the experiment acceptability criterion (Tables 1 and 4). Dissolved manganese did not cause coral tissue sloughing in any manganese treatment over the 14 d, therefore the NOEC was ≥1090 μg Mn/L which was the highest measured dissolved manganese treatment in the dilution series (Tables 4 and 5, Figs. 1 and 2b). The NOEC of ≥1090 μg Mn/L became 1090 μg Mn/L when used in the SSD as recommended by Warne et al. (2018) since it was not outside the range of existing toxicity data and did not have an overly large influence on the final GVs. There were no outward signs of manganese-induced stress to any of the corals in terms of mucus production or tentacle retraction, to the degree that was observed in the 2-d acute experiment (Fig. 2). Corals from the 500 and 1000 μg Mn/L treatments released more mucus than other corals when removed from the water at the end of the 14-d experiment but this was not visible when corals were in situ. There was no visual difference in colour intensity of corals exposed to manganese relative to the controls by 14 d, therefore no quantitative image analysis of coral bleaching was necessary. However, MFs were visible in corals in the 14-d chronic experiment, from 7 d onwards in all treatments other than the controls although there was no evidence of a concentration-response relationship between the degree of MF activity and dissolved manganese concentration (see Fig. S2 for examples of MFs).

MFs are used by corals to explore their surrounding environment when actively searching for food, as a defence mechanism against other marine biota (Nugues et al., 2004) and for secreting mucus and sterilizing wounds (Lewis et al., 2022) but their exact purpose when exposed to a contaminant is unknown. It is possible that the combined stressors of dissolved manganese and food limitation over the 14 d, triggered the corals to actively seek food with their MFs to replenish the energy spent on detoxifying the manganese. MF activity was not observed in the 2-d acute experiment where the effects of food limitation were not severe enough to trigger the action to seek food. The possible neurotoxic effects that caused tentacle retraction at the higher manganese concentrations in the 2-d acute experiment, may have also inhibited MF activity. In contrast, both tentacle retraction and MF activity were observed when *A. muricata* was exposed to manganese for 2 d but this also occurred in some of the control corals (Binet et al., 2023b). MF could not be used as a reliable endpoint of manganese effects in the 14-d experiment.

Because there were no differences in coral tissue sloughing with manganese concentration or between individual fragments, there were also no significant differences in manganese sensitivity between genotypes over the concentration range used (10–1000 μg Mn/L). This was in contrast to the acute experiment and the absence of outward signs of chronic toxicity at the whole organism level does not mean that there were no effects occurring at the subcellular 'omics' level in the chronic experiment that relate to the mechanism of manganese toxicity.

It was expected that based on the available acute EC50 data for other coral species, the chronic EC10 value would be closer to 80 μg Mn/L. This was calculated from the geometric mean of acute EC50 values for the three adult coral tissue sloughing endpoints which equated to 800 μg Mn/L, divided by a default ACR of 10. Logistical constraints meant that the chronic experiment for *A. millepora* in the current study was started before the acute experiment hence the acute EC50 value for *A. millepora* could not be used to inform the chronic experiment dilution series and it was unknown that *A. millepora* was more tolerant to manganese than the three other adult corals. However, future research on dissolved manganese chronic toxicity to *A. millepora* should focus on the concentration range between the 14-d NOEC (1090 μg Mn/L) and the 2-d EC10 (1300 μg Mn/L) values to determine the 14-d EC10 value for this species. This narrow concentration range suggests that the threshold where manganese transitioned from being an essential element to a toxic one was very close to the 14-d NOEC and therefore the chronic NOEC can be used with high confidence in the SSD for deriving the marine manganese GVs.

The coral-specific ACR of 2.3 was a factor of 4.3 times lower than the default ACR of 10 but given that *A. millepora* was more tolerant of dissolved manganese than the three other coral species, it would be useful in the future to compare the ACR derived from one of the three more sensitive coral species with the ACR for *A. millepora* to gauge how well it represents other coral species. The ACR may increase if other coral species are found to be more sensitive to chronic manganese exposure. The differences in manganese sensitivity between coral species and life stages is an important reminder that a single coral assay may not be representative of all coral species and all life stages.

3.5. Literature review and derivation of the marine manganese GVs

From the literature review and quality assessment process, there were 184 acute and chronic toxicity values that scored ≥50 % and were therefore categorized as 'acceptable' for further selection to ultimately be used in the SSD for GV derivation (Table S3). There were 105 acute values and 79 chronic values representing 24 and 29 species, respectively. Once the chronic toxicity values based on measured manganese and the acute coral EC50 values were selected, and one toxicity value was used to represent each species, there were 18 toxicity values representing five taxonomic groups (Cnidarians (corals), Molluscans (bivalves), Echinoderms (sea urchins), Ochrophytes (diatoms) and Haptophytes (microalga)), that comprised the final dataset for use in the SSD (Table 6).

The 18 values ranged in quality assessment scores from 70 to 96 % and consisted of three acute EC50 values converted to estimated chronic values for three adult corals using the coral-specific ACR derived in this study, five chronic NOEC values for a sea urchin, diatom, microalga and corals, and 10 chronic NEC values for bivalves. The coral (*A. muricata*), and sea urchin (*Heliocidaris tuberculata*), had more than one toxicity value for the same duration and endpoint, thus the geometric mean of the toxicity values for the respective species was used in the SSD (Table S3 and Table 6). A chronic NOEC value for gamete fertilisation was used to represent the coral *P. daedalea* rather than an available and lower EC10 value, as the reliability of the EC10 value was low according to Summer et al. (2019). The toxicity values ranged from 304 to 125,000 μg Mn/L with adult coral tissue sloughing being the most sensitive endpoint (estimated chronic NOEC of 304 μg Mn/L for *A. spathulata*) and coral gamete fertilisation being among the least sensitive endpoints (NOEC of 54,000 μg Mn/L for *P. daedalea*). Bivalve larval development ranged in sensitivity from NECs of 650 to 2410 μg Mn/L while the diatom and microalga were very tolerant with NOECs of 18,000 and 125,000 μg Mn/L, respectively.

The data distribution was assessed for modality or sub-groups within the data that may warrant separate SSDs, using a weight-of-evidence approach according to Warne et al. (2018). The data were not considered to be bimodal but did have a right-skewed tail because the majority of the data ranged from 304 to 2410 μg Mn/L while the remaining values were much higher at 18,000–125,000 μg Mn/L. There was no one

Table 6
Dissolved (<0.45 μm filtered) manganese toxicity data for marine species used in the species sensitivity distribution.

Taxonomic group	Species	Life stage	Duration (h)	Type (acute/ chronic)	Toxicity measure	Final toxicity value (μg/L)	Reference
Cnidarian (coral)	*Acropora spathulata*	Adult	48	Acute	EC50 (tissue sloughing)	304[a]	Summer et al. (2019)
Cnidarian (coral)	*Acropora muricata*	Adult	48	Acute	EC50 (tissue sloughing)	358[a,b]	Binet et al. (2023b)
Cnidarian (coral)	*Stylophora pistillata*	Adult	48	Acute	EC50 (tissue sloughing)	374[a]	Stauber et al. (2002)
Bivalve (oyster)	*Magallana gigas*[c]	Embryo	48	Chronic	NEC (embryo development)	650	Markich (2021)
Bivalve (oyster)	*Saccostrea glomerata*[d]	Embryo	48	Chronic	NEC (embryo development)	654	Markich (2021)
Bivalve (mussel)	*Xenostrobus securis*	Embryo	48	Chronic	NEC (embryo development)	755	Markich (2021)
Bivalve (scallop)	*Scaeochlamys livida*	Embryo	48	Chronic	NEC (embryo development)	959	Markich (2021)
Bivalve (cockle)	*Anadara trapezia*	Embryo	48	Chronic	NEC (embryo development)	1040	Markich (2021)
Cnidarian (coral)	*Acropora millepora*	Adult	336	Chronic	NOEC (tissue sloughing)	1090	Current study
Bivalve (cockle)	*Fulvia tenuicostata*	Embryo	48	Chronic	NEC (embryo development)	1460	Markich (2021)
Bivalve (clam)	*Hiatula alba*	Embryo	48	Chronic	NEC (embryo development)	1520	Markich (2021)
Echinoderm (sea urchin)	*Heliocidaris tuberculata*	Embryo	72	Chronic	NOEC (embryo development)	1,580[b]	Levy et al. (2004), ESA (2008)
Bivalve (clam)	*Barnea australasiae*	Embryo	48	Chronic	NEC (embryo development)	1780	Markich (2021)
Bivalve (clam)	*Spisula trigonella*	Embryo	48	Chronic	NEC (embryo development)	2090	Markich (2021)
Bivalve (clam)	*Irus crenatus*	Embryo	48	Chronic	NEC (embryo development)	2410	Markich (2021)
Ochrophyte (diatom)	*Ceratoneis closterium*[e]	N.A.	72	Chronic	NOEC (growth rate)	18,000	Levy et al. (2004)
Cnidarian (coral)	*Platygyra daedalea*	Gametes	5.5	Chronic	NOEC (fertilisation)	54,000	Summer et al. (2019)
Haptophyte (microalga)	*Tisochrysis lutea*[f]	Log phase	72	Chronic	NOEC (growth rate)	125,000	ESA (2008)

Values are reported to no more than three significant figures. For all data that score ≥50 % refer to Table S3.
N.A data was not available.
[a] Estimated chronic toxicity value derived by dividing the acute EC50 by a coral ACR of 2.3.
[b] Geometric mean of several values.
[c] Formerly known as *Crassostrea gigas*.
[d] Formerly known as *Saccostrea commercialis*.
[e] Formerly known as *Nitzschia closterium*.
[f] Formerly known as *Isochrysis galbana* or T-ISO.

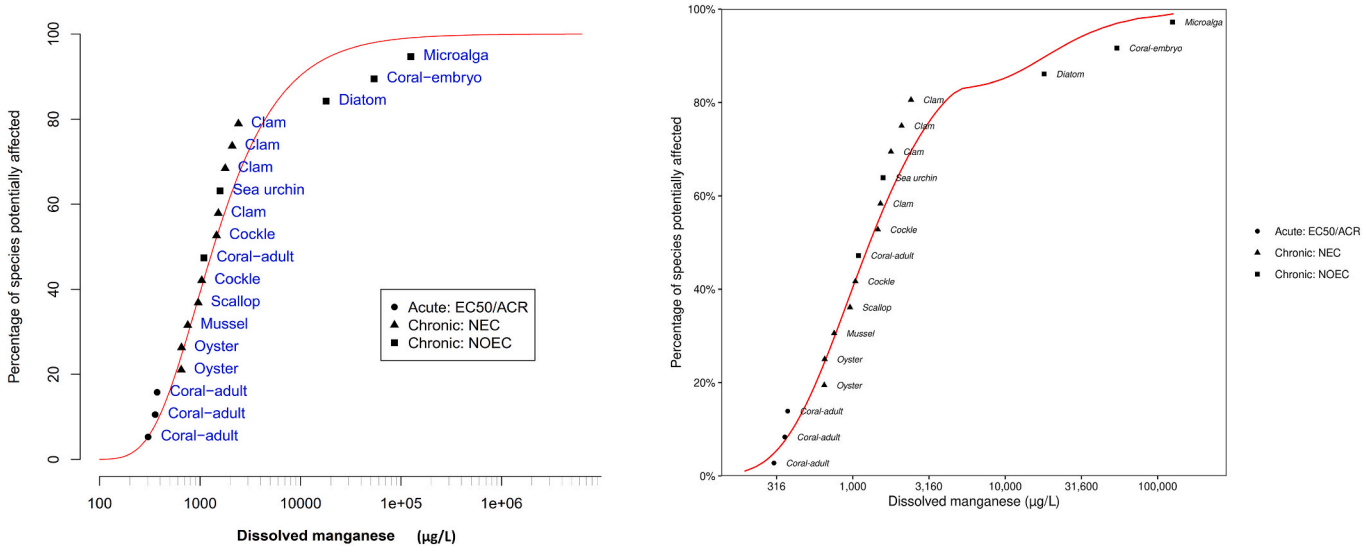


Fig. 3. Species sensitivity distributions plotted using Burrlioz (left) and shinyssdtools (right) software for chronic toxicity of dissolved manganese to marine biota.

taxonomic group that was more sensitive than the others, however, there was a strong difference in coral sensitivity to dissolved manganese related to life stage, with adult corals being more sensitive than early life stages and hence the toxicity values for coral ranged from 304 to 54,000 μg Mn/L. All 18 values were used in the SSD.

An inverse Weibull model was fitted to the 18 chronic and acute

Table 7
Guideline values (GVs) for dissolved (<0.45 μm filtered) manganese in marine waters for 80, 90, 95 and 99 % of species protection using Burrlioz and Shinyssdtools.

Protection level	Burrlioz GVs (μg Mn/L)	Shinyssdtools GVs (μg Mn/L)
High conservation value systems (99 % species protection, PC99)	190	200
Slightly to moderately disturbed systems (95 % species protection, PC95[a])	300	310
Highly disturbed systems (90 % species protection, PC90)	390	400
(80 % species protection, PC80)	570	570

[a] PC95 is also known as the HC5. Values are shown with two significant figures.

(converted to chronic) toxicity values using the recommended Burrlioz software to derive the SSD (Warne et al., 2018) (Fig. 3). The model fit was assessed as being good, especially at the mid to lower end of the curve where the concentration that protects 95 % of species (PC95), otherwise known as the hazardous concentration to 5 % of species (HC5), is determined. From the SSD, the GVs that provide 99, 95, 90, and 80 % of species protection from dissolved manganese in marine waters were 190, 300, 390, and 570 μg Mn/L, respectively (Table 7). According to the decision matrix from Warne et al. (2018), our dataset was of a "preferred dataset size" of 18 values that were a combination of chronic and acute (converted to chronic) values, with a good SSD model fit which put these GVs into the category of 'moderate' reliability. This is a significant improvement from the current low-reliability interim working level of 80 μg Mn/L (ANZG, 2018). The PC95 value of 300 μg Mn/L, is commonly used for the long-term protection of slightly to moderately disturbed marine ecosystems and is very similar to the lowest estimated chronic toxicity value of 304 μg Mn/L for adult *A. spathulata* tissue sloughing which indicates that these GVs will be protective of the most sensitive species to manganese. The GVs are also well above the background concentration of dissolved manganese in seawaters globally which ranges from 0.004 to 0.38 μg Mn/L (Table S1) and the analytical detection limit for commercial laboratories (LOD < 1 μg Mn/L). This means that no corrections to the GVs will be necessary for naturally elevated background dissolved manganese and the LODs are sufficiently low to measure dissolved manganese concentrations accurately at the GVs. Interestingly, the PC95 value (300 μg Mn/L) is the same as that derived by WHO (2004) using an SSD approach with a different dataset that excludes adult corals, and is two times higher than the site-specific marine manganese GV of 140 μg Mn/L derived by Stauber (2006) using an SSD approach and one adult coral species in the dataset. Overall, the GVs from this study will be protective of the most sensitive marine organisms including coral, and less conservative than the previous low-reliability interim working level.

Because the true NOEC for *A. millepora* may be higher than 1090 μg Mn/L which would lower the ACR, GVs derived with lower ACRs of 1.5 (i.e., a NOEC of 1710 μg Mn/L), and 2.0 (i.e., a NOEC equivalent to the 2-d EC10 of 1300 μg Mn/L) were compared to those GVs based on an ACR of 2.3 to determine the influence of using the NOEC as 1090 μg Mn/L on the GVs (Table S5). There was very little influence of these lower ACRs on the GVs. GVs at all levels of protection increased by factors of 1.1 to 1.4 and comparable GVs had overlapping 95 % confidence limits. However, the use of the PC99 (i.e., HC1) value of 270 μg Mn/L (based on an ACR of 1.5) rather than the PC95 would be required to protect more sensitive coral species such as *A. spathulata*. These comparisons support the use of the NOEC as 1090 μg Mn/L for deriving the GVs and recognise the need for more chronic toxicity data for adult corals.

The majority (56 %) of toxicity values in the dataset were for bivalves that came from one publication by Markich (2021) and encompassed the median, upper and lower quartiles of the final dataset. The influence of these bivalve values on the fit of the SSD and GVs derived from Burrlioz was investigated by removing them from the dataset and re-examining the SSD and GVs (Table S6, Fig. S4). The inclusion of these values in the final toxicity dataset increased the number of taxonomic groups represented from four to five, improved the fit of the SSD, raised the GVs by no more than a factor of 2.4 and did not change the GV reliability status. Therefore, the bivalve toxicity data made an important contribution without having undue influence on the final GVs.

An alternative software package for fitting an SSD model to the final 18 chronic manganese toxicity values to derive GVs was the shinyssdtools web-based freeware. This approach involved fitting the six default models (gamma, lgumbel, llogis, lnorm, lnorm_lnorm, and weibull, Table S7, Fig. S5), simultaneously and using the model average as the best fitting SSD to derive the PC99, PC95, PC90, and PC80 which were 200, 310, 400 and 570 μg Mn/L, respectively (Table 7, Fig. 3). The weighting and statistics of best fit for each of the models showed that the lgumbel and lnorm_lnorm models had the highest weightings, and best fit statistics while the gamma and weibull models had no weighting and poor fit statistics (Table S7). Alternative models to the default options were also trialled but were too similar to the existing default models in their fits and so were removed to prevent similar models from artificially inflating the weighting. The GVs derived using shinyssdtools were not considered to be significantly different from those derived using Burrlioz. The advantage of using the model average approach is that a better fit of the SSD model to a small dataset can be achieved (Binet et al., 2023a; Fox et al., 2022). Evidence of the better fit of the average model approach can be seen by the shape of the SSD from shinyssdtools which has a better visual fit at the upper end of the curve than the Burrlioz SSD (Fig. 3). However, in this case, the SSD fits at the lower end of the curve were similar for both software packages and consequently the final GVs and their status of reliability were the same. Burrlioz is the recommended software package to use for default GV derivation in Australia and New Zealand at this time (Warne et al., 2018).

4. Conclusion

This study provides the first acute and chronic manganese toxicity data for the sensitive biological endpoint of tissue sloughing for the adult reef-building scleractinian coral, *A. millepora*. The manganese speciation measurements confirmed that *A. millepora* was exposed to operationally defined truly dissolved, rather than colloidal or precipitated manganese and so was responding to the most bioavailable form of manganese. The acute 2-d EC50 value of 2560 μg Mn/L for adult coral tissue sloughing showed that *A. millepora* was 3–4 times more tolerant to dissolved manganese than comparable data for three other coral species, suggesting there are small interspecies differences in the sensitivity of adult coral to dissolved manganese relative to the difference in sensitivity between coral life stages. There were also differences between genotypes of *A. millepora* in their sensitivity to manganese as the concentration increased and the potential sensitivity bias associated with genotype should be factored into experimental designs with corals. The acute

toxicity data from *A. millepora* provides further evidence that the adult life stage of coral is more sensitive than the early life stage to manganese and that bleaching does not occur prior to tissue sloughing.

The chronic 14-d EC10 value for dissolved manganese effects on adult *A. millepora* tissue sloughing could not be determined, however in the absence of a concentration-response, the highest concentration (1090 µg Mn/L) in the dilution series was used as a NOEC. It is likely that the chronic EC10 value lies in the narrow concentration range of 1090 to 1300 µg Mn/L. The ratio of the acute EC50 and chronic NOEC for *A. millepora* became a coral-specific ACR of 2.3 to convert the acute data for other coral species that currently exists to estimated chronic toxicity data. The chronic toxicity of dissolved manganese to adult corals, based on tissue sloughing for four coral species, ranged from 304 to 1090 µg Mn/L which is generally higher than the current range in manganese concentrations for contaminated seawaters, with the exception of catastrophic events. However, adult corals are among the most sensitive marine organisms to manganese and in a multi-stressor marine environment, the effects of elevated dissolved manganese may be compounded. Further research on the chronic toxicity of dissolved manganese to *A. millepora* and other more sensitive coral species using high flow-through systems as in this study, is needed in the future to investigate other early warning sublethal endpoints such as 'omics' and mucus responses, and determine the mechanism of toxicity that results in adult coral tissue sloughing.

The chronic NOEC value for adult *A. millepora* and estimated chronic values for other adult corals were combined with high quality chronic toxicity data from the literature for marine species in an SSD to determine water quality GVs at different levels of ecosystem protection. The use of two different SSD software packages (Burrlioz and shinyssdtools) gave similar GVs despite using different modelling approaches. Marine manganese GVs were 190, 300, 390 and 570 µg Mn/L to provide long-term protection of 99, 95, 90, and 80 % of marine species, respectively. These water quality GVs were less conservative and more reliable than the interim low-reliability value currently available and will provide long-term protection for sensitive coral and other marine organisms as the demand for manganese and associated critical elements grows in the future.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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