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Assessing the chronic toxicity of copper and aluminium to the tropical sea anemone Exaiptasia pallida

Melanie A. Trenfield[a,b,c,*], Joost W. van Dam[b], Andrew J. Harford[c], David Parry[d,e], Claire Streten[b], Karen Gibb[a], Rick A. van Dam[c]

[a] North Australian Marine Research Alliance, Charles Darwin University, Darwin, Northern Territory 0909, Australia
[b] Australian Institute of Marine Science, 23 Ellengowan Dve, Brinkin, Northern Territory 0811, Australia
[c] Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin, NT 0801, Australia
[d] Rio Tinto Aluminium, 123 Albert St, Brisbane, Queensland 4000, Australia
[e] Charles Darwin University, Darwin, Northern Territory 0909, Australia

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ABSTRACT

The world's most productive bauxite mines and alumina refineries are located in tropical or sub-tropical regions. The discharge water from alumina refineries can contain elevated aluminium (Al, < 0.45 μm fraction), from 30 to 1000 μg/L. There is a need for additional information on the toxicity of Al to aquatic organisms to improve the environmental regulation and management of alumina refinery operations in tropical coastal regions. A 14-d chronic toxicity test was developed for the tropical sea anemone *Exaiptasia pallida*. Asexual reproduction and growth rates of *E. pallida* were assessed using the number of lacerates produced and oral disc diameter. The comparative sensitivity of *E. pallida* was assessed through exposure to a commonly-used reference toxicant, copper (Cu) at 28 °C, with asexual reproduction toxicity estimates of 10% (EC10) and 50% (EC50) effect concentrations, calculated as 8.8 μg/L (95% confidence limits (CL): 1–18 μg/L) and 35 μg/L Cu (95% CL: 30–39 μg/L), respectively. Growth rate was a suitable additional endpoint (EC50 = 35 μg/L Cu, 95% CL: 23–49 μg/L). The EC10 and EC50 for Al (total fraction, based on reproduction) at 28 °C were 817 μg/L (95% CL: 440–1480 μg/L) and 2270 μg/L (95% CL: 1600–3900 μg/L), respectively. The toxicity of Cu and Al was also assessed at 24 °C and 31 °C, representing average year-round water temperatures for sub-tropical and tropical Australian coastal environments. Changing the temperature from 28 °C to 24 °C or 31 °C resulted in up to 45% less reproduction of anemones and increased their sensitivity to Cu (EC50s at 24 °C = 21 μg/L, 95% CL: 17–26 μg/L and at 31 °C = 23 μg/L, 95% CL: 21–25 μg/L). Sensitivity to Al was reduced at 24 °C with an EC50 of 8870 μg/L (95% CL: 6200-NC). An EC50 for Al at 31 °C could not be calculated. This test is a reliable and sensitive addition to the suite of standardised tests currently developed for tropical marine species.

1. Introduction

Tropical marine systems worldwide are becoming highly impacted, with point source pollution considered one of the greatest threats (Halpern et al., 2007, 2008). One such source of pollution is effluent from coastal mining and mineral processing operations. Bauxite reserves and mines are typically located in tropical and subtropical regions (Alumina Limited, 2016; Aluminum Association, 2016; Rio Tinto Alumina, 2016). While surface water concentrations of dissolved aluminium tend to range between 1–5 μg/L, in Australia, concentrations above 50 μg/L total Al have been measured from surface water in close proximity to alumina refineries and an aluminium smelter (Angel et al., 2012). The environmental risk of such mines and associated alumina refineries is mitigated through regulation of the industry and the associated use of Guideline Values (GVs) for contaminants of concern that inform their management (ANZECC and ARMCANZ, 2000; CCME, 2003; WFD UK, 2007; USEPA, 2012). However, for many contaminants in marine water there are no GVs available and, where GVs do exist, they are largely based on toxicity data from temperate species and such is the case for Al (van Dam et al., 2008; Golding et al., 2015). This is due to a shortage of regionally relevant chronic toxicity data (van Dam et al., 2008), resulting from a lack of standardised test methods for tropical marine species. Insufficient representation of tropical species in the derivation of existing GVs may lead to inadequate protection for tropical marine biota, which may respond differently to stressors than their temperate counterparts (Kwok et al., 2007; Rico

* Correspondence to: Department of the Environment and Energy, Eaton, Northern Territory 0820, Australia.
E-mail address: melanie.trenfield@environment.gov.au (M.A. Trenfield).

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et al., 2011). A more comprehensive assessment of the toxicity of Al to tropical marine species is needed to improve environmental regulation and management of alumina refinery operations in tropical coastal regions.

The sea anemone, *Exaiptasia pallida*, formerly known as *Aiptasia pulchella* (Grajales and Rodriguez, 2014), was selected for this study due to its widespread distribution in tropical and subtropical shallow-water marine environments (Brock and Bielmeyer, 2013). Sea anemones have been found to naturally occur in the Arafura Sea, an area of alumina refinery discharge in northern Australia (Department of the Environment & Energy, 2005). *Exaiptasia pallida* is very easily cultured in aquaria, and previous toxicity studies have indicated that it is sensitive to the ubiquitous toxicant Cu (Mitchelmore et al., 2003; Main et al., 2010; Brock and Bielmeyer, 2013; Howe et al., 2015). A Cnidarian (along with jellyfish, hydra and coral), *E. pallida* has been shown to have similar sensitivity to Cu as that of corals (Howe et al., 2012). As such, anemones have been suggested as a proxy for testing the effects of contaminants on corals (Madin, 2011; Howe et al., 2012), as coral toxicology is restricted by seasonal availability of organisms from the wild. Reproduction of *E. pallida* can occur asexually by pedal laceration, whereby fragments of tissue are pinched off from the basal disc. A chronic test method developed for this species by (Howe et al., 2012, 2015) suggested asexual reproduction could be a useful indicator of environmental change and serve as a suitable test endpoint. However, there were some aspects of the test design by Howe et al. (2012) that prompted further development, including establishing a greater reproductive rate for control anemones.

The aims of this study were to: a) optimise a routine chronic toxicity test method to assess the effects of contamination on reproduction and growth of *E. pallida* and b) investigate the toxicity of Cu and Al at 24, 28 and 31 °C. Copper toxicity was assessed in order to provide a comparison of sensitivity with other species and because of its widespread nature as a contaminant associated with urban activity.

2. Materials and methods

2.1. Exaiptasia pallida culture

Exaiptasia pallida was harvested from a population which established naturally in the aquaria at the Australian Institute of Marine Science in Darwin, Northern Territory, Australia. After its potential suitability as a test species was identified, individuals were separated to a specific system for ease of maintenance and selection of individuals for tests. Around 400 adults (~0.3 – 15 mm oral disc diameter) were maintained in 20 L of filtered seawater (FSW, 0.5 µm) in a 30 L HDPE container, connected to a recirculating system in a temperature controlled room. Several air stones were spaced evenly around the tank to provide aeration, and the tank was kept free of rocks or additional substrate so that the anemones could attach only to the tank surface. Broodstock were maintained in this aquaria over the course of testing (~ one year) at pH 8.3 ± 0.1, 29.5 ± 0.6 °C, salinity of 34.6 ± 0.3 PSU (conductivity =51.8 ± 1 mS/cm) and with a 12:12 h day/night cycle (representative of the diurnal light cycle in northern Australia, indirect 36 W cool white triphosphor ceiling lighting which was measured as ~ 2–3 µmol photons/m^2/s inside the enclosed tank). Broodstock were fed live brine shrimp (*Artemia salina*) each Monday, Wednesday and Friday, harvested from a 1 L Imhoff cone. The *A. salina* was prepared by adding 1 teaspoon of cysts to 1 L of FSW. The culture was aerated for 24 h under cool white light with a 12:12 h day/night cycle.

2.2. Preparing anemones for testing

The anemone culture was fed *A. salina* on the Monday morning of the start of the test, using the method described above. After allowing time for the food to be consumed (~four h), anemones were transferred to test solutions.

Adults (of either 5 or 10 mm oral disc diameter) were removed from the culture tank by gently scraping them from the tank surface using a cell scraper (TPP, blade width 13 mm). Adults were then transferred into the test solutions and observed ~15 min later under a microscope to check that all individuals were normal and healthy, and to remove any debris or attached lacerates which may have been transferred with the adults. All anemones were transferred to test solutions at 28 °C and the test containers then transferred to incubators at either 24 °C, 28 °C or 31 °C. For those tests being run at 24 °C and 31 °C, it is estimated that the acclimation period for the anemones in the 300 mL volume would have occurred over 4–5 h.

2.3. Optimising test conditions

Initial 14-d trials conducted in 0.45 µm filtered seawater focused on optimising several features of the method including replicate volume (300 vs 500 mL), container shape (round vs rectangular), number of anemones per replicate (5 vs 10), anemone size (~5 mm vs 10 mm) and feeding frequency (daily vs 3x/week).

2.4. Chemicals and equipment

All equipment that came into contact with *E. pallida*, control water or test solutions were made of chemically inert materials (e.g. silicon, glass or polyethylene). All plastic and glassware were soaked in 5% v/v nitric acid (HNO_3, Chem-supply) for 24 h before rinsing with deionised, reverse-osmosis water (Elix, Millipore). All reagents used were analytical grade and stock solutions were made using high purity water (18 MΩcm, Milli-Q, Millipore).

2.5. Preparation of test solutions

Seawater was collected at high tide from Nightcliff jetty, Northern Territory, Australia (12°22'59"S, 130°50'56"E) and passed through a 0.45-µm filter immediately following transport of the water to the laboratory (Quickfilter groundwater cartridge, Thermofisher Scientific). Stock solutions of 20 and 2000 mg/L for each metal were prepared by dissolving $CuSO_4{\cdot}2H_2O$ and $AlCl_3{\cdot}6H_2O$ (Sigma-Aldrich) in Milli-Q water. Test solutions (nominal metal concentration ranges Cu: 0–70 µg/L and Al: 0–10000 µg/L) were prepared by adding stock solutions to filtered seawater, and were stored in 5 L HDPE bottles for 24 h prior to test initiation to allow for equilibration. Test solution bottles were stored in a refrigerator throughout the test period and removed only to dispense new test solutions on each water change day.

2.6. Physico-chemical analyses

The dissolved oxygen (DO), pH, conductivity and salinity of each treatment were measured at test commencement (0 h), with renewal waters and 48–72-h old waters of each treatment measured each Monday, Wednesday and Friday of the 14-d exposure (Hach multiprobe HQ40d). Incubator temperatures and light intensity were logged every 10 min using Hobo data loggers (Hoboware). At 0 h, test solutions from Cu exposures were sub-sampled for the < 0.45 µm fraction and solutions from Al exposures were sub-sampled unfiltered for analyses of total concentrations of selected metals, and acidified to 0.13% nitric acid (HNO_3, AR grade, Crown Scientific). All samples were analysed for Al, Cd, Co, Cu, Fe, Ga, Mo, Ni, Pb and Zn using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Agilent 7700 ce). Filtered sub-samples (< 0.45 µm fraction representing concentrations of dissolved Al) were taken only for the first four Al exposures. This was discontinued following determination that the required concentration range would exceed the solubility limit of Al in seawater (~500 µg/L, Angel et al., 2016). For several tests for both Cu and Al, sub-samples were also taken from 72-h old test solutions at the end of the 14-d test period and