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The sensitivity of embryos and larvae of the crown-of-thorns sea star to copper and zinc: with respect to conditions experienced on the Great Barrier Reef and water quality guidelines

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ABSTRACT

Our understanding of the ecotoxicology for tropical marine species is limited. We investigated the impacts of copper (0.1–6 μg/L) and zinc (2.5–45 μg/L) on development of the crown-of-thorns sea star (COTS) (*Acanthaster* sp.) with respect to lethal concentration (LC) and effective concentration (EC) for inhibition of larval swimming and in context with concentrations reported for the Great Barrier Reef (GBR) and the Australia and New Zealand Water Quality Guidelines (WQG). For embryos, copper caused 100 % mortality by 24 h and zinc arrested development by 36 h. The LC50s for bipinnaria for copper at 24 and 48 h were 0.67 and 0.54 μg/L and for zinc were 52.36 and 27.01 μg/L, respectively. For brachiolaria, these were 2.82 and 0.85 μg/L and 28.89 and 22 μg/L, respectively. For larval swimming the EC50 for copper was 0.35 μg/L and 0.66 μg/L in bipinnaria and brachiolaria, respectively. Larvae placed in copper (0.1–0.2 μg/L) recovered their swimming ability. Bipinnaria were resistant to zinc, EC50 of 28.89 μg/L for swimming, while for brachiolaria this was 7.18 μg/L. *Acanthaster* development was sensitive to copper and zinc at levels reported for nearshore GBR waters. This may contribute to the general absence of COTS on inshore reefs. Our data showed that the 95 % species protection for copper and zinc (1.3 μg/L and 15 μg/L, respectively) are not suitable for COTS development. This is likely to apply to many other species with similar planktonic larvae in GBR waters. This study will contribute to development of WQG for tropical marine waters.

1. Introduction

Marine invertebrate embryos and larvae are highly sensitive to metal contaminants as shown by the high efficacy of biocidal antifouling coatings on ships hulls (Haynes and Loong, 2002). The high sensitivity of marine invertebrate development to pollutants even in very low concentrations also underlies their use as a model system to determine water and sediment quality guidelines (US EPA, 1995; ANZG, 2018). Regulatory tests and effluent toxicity screening often involves the gametes and developmental stages of molluscs, echinoderms, and algae (Okazaki, 1976; Martin et al., 1981; Macfie et al., 1994; Stauber et al., 1996) with most national marine water quality guidelines based on toxicology data from temperate species (e.g., ANZG, 2018). Far less is known about impacts of pollutants on the development of tropical marine invertebrates, despite their great diversity, data are only available for a few species (Reichelt-Brushett and Hudspith, 2016; Gissi et al., 2018; Markich, 2021).

One of the best studied tropical marine ecosystems is the World Heritage, Great Barrier Reef (GBR), Australia where water quality has long been a concern with respect to reef health (Brodie et al., 2012; Kroon et al., 2020;2023). Water quality has declined since the establishment of agriculture and farming in river catchments that inflow into the GBR lagoon in the 1800′s (Kroon et al., 2020,2023). Runoff from coastal agriculture and farming have significantly reduced the quality of in-shore waters and this is exacerbated by large runoff events from major river systems that occur in association with cyclones and monsoonal rainfall (Brodie et al., 2012; Devlin et al., 2012). These events are common in the northern GBR where they create large plumes of low salinity water that can extend a distance across the reef (Devlin and Schaffelke 2009; Brodie et al., 2012). Other land-based sources of pollution in the region include urban runoff, waste treatment and disposal, mining and other industry and defence activities (Bartley et al., 2017; Gissi et al., 2018; Kroon et al., 2020;2023). Inner-shelf reefs on the GBR are exposed regularly to a cocktail of dissolved nutrients,

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herbicides, and pesticides in river plumes (Devlin and Schaffelke 2009; Kroon et al., 2020). Water quality improvement, to reduce land runoff is a major management priority (GBRMPA, 2010). This is also part of the of the Reef 2050 Long Term Sustainability Plan (GBRMPA, 2021).

In addition to land-based pollutants, boating and maritime activities are also a source of heavy metal pollution in GBR waters (Kroon et al., 2020). Antifouling systems, coating used to control or prevent attachment of biota on ships hulls contain metal oxides including copper and zinc. Vessels underway and at anchor can leach up to 0.8–3.2 kg/per day of these metals (PGM Environment, 2012). With respect to Australian marine quality guidelines (ANZG, 2018), exceedance of contaminant levels for 95 % species protection in water and sediment have been recorded for many chemicals and metals at locations in and adjacent to the GBR (Kroon et al., 2020). For copper and zinc the guideline trigger values for deleterious effects are 1.3 μg/L and 15 μg/L, respectively (ANZG, 2018). Higher levels have been recorded in reef waters (Kroon, 2020, see Supplementary Table 1).

Despite the importance of tropical marine waters across the globe with coastal development alongside high biodiversity systems, the ecotoxicology of tropical marine species is poorly studied (Gissi et al., 2018). For instance, most research on the impacts of pollution on the GBR to marine invertebrates has focused on corals (Howard et al., 1986; Equivel, 1986; Reichelt-Brushett, 1999; Negri and Heyward, 2001; Reichelt- Brushett and Hudspith, 2016; Binet et al., 2023), with a recent study from northern Australia also incorporating a gastropod, a copepod and a crustacean (Gissi et al., 2018). Although copper and zinc are trace metals that occur in low concentrations in healthy marine organisms, high concentrations of these metals as often found in the marine environment can exert sub-lethal and lethal effects (Kroon et al., 2020). Exposure of coral polyps to copper at concentrations >2 μg/L can result in photosynthesis reduction of algal symbionts and changes in oxygen consumption (Bielmyer et al., 2010). With respect to coral gametes and development, copper (>17.4 μg/L) and zinc (>96 μg/L) can impair or inhibit fertilisation, larval motility, larval settlement, and metamorphosis (Reichelt-Brushett and Harrison 1999; Negri and Heyward, 2001; Reichelt- Brushett and Hudspith, 2016). Negative effects of copper and zinc on marine invertebrate development is widely reported, largely in studies of temperate mollusc and echinoderm species (Heslinga 1976; Kobayashi, 1980; Castagna et al. 1981; Ramachandran et al. 1997; King and Riddle, 2001; Dermeche et al. 2012; Holan et al., 2016; Cunningham et al., 2020; Markich, 2021).

Due to the high sensitivity of their gametes and development, echinoderms are used routinely as model species for ecotoxicology testing of marine contaminants in many jurisdictions (e.g. US EPA, 1995). Ecological studies show that sensitivity to environmental stressors varies between species, developmental stage, larval type and habitats (Byrne, 2012; Martino et al., 2017; Balogh and Byrne, 2020). Thus, it is important to consider species-specific and developmental stage-specific sensitivities with respect to the impacts of contaminants on regional biodiversity and in application of water quality guidelines. Sensitivity can vary greatly even between closely related species that occur in different latitudes and habitats (Martino et al., 2017).

In Australia, water quality guidelines are based on analysis of global data for adults of temperate species (north and southern hemisphere), incorporating 70 data points for copper and data from five taxonomic groups, with no echinoderms included (Australia and New Zealand Water Quality Guidelines - ANZG, 2018). Temperate species are likely to have different sensitivities to toxicants than related tropical species (Chapman and Riddle 2005). There is a paucity of information on the impacts of trace elements on the development of tropical echinoderms with no data for any species that occur on the GBR (Markich, 2002; ANZG, 2018), an important knowledge gap with respect to concerns on contaminants entering the GBR lagoon (Kroon et al., 2020). We address this here in an investigation of the impacts of cooper and zinc on embryo and larval development in the ecologically influential species, the crown-of-thorns sea star (COTS, *Acanthaster* sp.)

As the most important coral predator and a key driver in the decrease in coral cover on Indo-Pacific reefs, COTS has been of great research interest (Pratchett et al., 2017; Deaker and Byrne 2022). We investigated the impacts of a range of copper and zinc concentrations (copper: 0.1–6 μg/L; zinc: 2.5–45 μg/L) on development of this species in consideration with concentrations of these metals reported for GBR waters and in context with species protection trigger guideline values for these metals (ANZG, 2018). As sensitivity to environmental stressors varies through development, we investigated the tolerance of three developmental stages of COTS including embryos and two larval stages, bipinnaria and brachiolaria. It is also important to consider the sensitivity of these stages with respect to their location in the benthos (embryos) or in the water column (larvae). Embryos are less likely to encounter contaminants in surface plumes of water containing copper and zinc following a runoff event or due to leaching from ship hulls.

We determined the survival and development of embryos and larvae as well as larval swimming ability in a range of copper and zinc concentrations. The LC50 (lethal concentration; 50 % survival) and EC50 (50 % effect on a non-lethal trait) were determined as in previous ecotoxicology studies of a range of species (Heslinga 1976; Kobayashi, 1980; Castagna et al. 1981; Ramachandran et al. 1997; King and Riddle, 2001; Dermeche et al. 2012; Holan et al., 2016). We compared our results with that determined for the embryos and larvae of other echinoderms with virtually all data from temperate sea urchin species (Kobayashi, 1980; Dermeche et al., 2012). As there are no ecotoxicology studies on the impacts of copper and zinc on the development of tropical sea stars, this study also provides a guide as to the sensitivity of a diversity of co-occurring species that have larvae similar to those of COTS (e.g. *Linckia*), many of which have a broad Indo-Pacific distribution. Thus, this study contributes to the development of water quality guidelines for tropical marine waters.

We predicted that deleterious impacts would be evident for copper at a lower concentration than for zinc as determined for sea urchin embryos and larvae (e.g. Kobayashi, 1980; Demerche et al., 2012). It was expected that threshold concentrations for deleterious effects would change over the length of exposure. As *Acanthaster* appears to be a stress tolerant species across life history stages (Uthicke 2013; Kamya et al. 2016, 2018; Lang et al. 2022; Byrne et al., 2023, but see Clements et al., 2022), tolerance to copper and zinc was expected to be higher than predicted by the 95 % species protection guidelines for these metals (ANZG, 2018), however high mortality in the high concentration treatments was anticipated.

2. Methods

2.1. Animal collection, spawning and rearing

Acanthaster sp. were collected from the Great Barrier Reef (GBR) near Cairns, Queensland by the COTS control team in November 2020 and shipped to the Sydney Institute of Marine Science. As the taxonomy of the Pacific species of *Acanthaster* is uncertain (Haszprunar and Spies 2014), we refer to the species on the GBR as *Acanthaster* sp. or COTS. The animals were kept in flow-through aquaria at 26 °C, the temperature of the collection site. For two separate fertilisations portions of the ovary and testes were removed from two different females and two different males. The ovaries were rinsed in filtered sea water (FSW, 1μ M) and placed in a 10^{-5} M 1-methyladenince solution to induce ovulation. Sperm were stored dry in plastic tubes at room temperature for 20 min before use. For each fertilisation, eggs from the two females were combined in equal proportions in beakers of FSW at 26 °C, the temperature that the gametes and developmental stages experience on the GBR. Similarly, sperm were combined in equal proportions from each male in fresh FSW. Eggs were then fertilised (~ 10^5 sperm/ml) at 26 °C and three counts of 100 individuals indicated ≥ 95 % fertilisation). For the embryo tests, once cleavage was observed on microscopic examination, they were used for the experiment. Separate larval cultures were reared for the larval tests. Larvae were reared at 26 °C in 1 L beakers, supplied with new FSW every second day with renewal by reverse aspiration. The larvae were fed every second day with *Proteomonas sulcuta* (30 × 10^3 cell ml^{-1}) determined using haemocytometer counts in parallel with water change from 72 h post fertilisation. Larvae were reared to the bipinnaria and brachiolaria stages for use in experiments (see below).

2.2. Metal concentrations

The impact of exposure to copper or zinc on the developmental stages; cleavage, bipinnaria and brachiolaria were assessed under a static non-renewal test regime. Stock solution of 300 μg/L $CuSO_4$ and 300 μg/L $ZnCl_2$ were made in FSW and a serial dilution of each was made in FSW to achieve a range of concentrations for testing. Nine concentrations were made for copper (0.1, 0.2, 0.3, 0.5, 1, 1.5, 3, 6, 12 μg/L) and seven concentrations were made for zinc (2.5, 5, 7.5, 15, 30, 45 μg/L). These concentrations include the levels considered to achieve 99 %, 95 %, 90 % and 80 % species protection according to marine water quality guidelines used for regulation (ANZG, 2018). For copper these concentrations are 0.3,1.3, 3 and 8 respectively and for zinc are 7, 15, 23 and 43 respectively (ANZG, 2018).

A large concentration range of the two metals was used to identify levels for LC50 (lethal concentration ≤ 50 % survival) as well as the EC50 for a non-lethal trait which indicates effective concentrations with ≥50 % survival. For the latter parameter we scored abnormal development for embryos and swimming ability for bipinnaria and brachiolaria (see below).

2.3. Test procedures

Tests were carried out in 10 mL borosilicate glass vials held at constant temperature (26 °C) in a water bath or constant temperature room. All vials were rinsed with FSW before use. Five vials were used for each metal concentration (see below) at each developmental stage with 15 individuals (embryos or larvae) per vial. Controls (FSW) were run in parallel for each test. We used new vials for each test.

Water quality parameters were measured before scoring. Dissolved oxygen (DO) was measured with an optical DO probe (Vernier Software and Technology, USA) and remained above 90 %. Salinity and pH were measured by a WTW Multimeter. For copper treatments the salinity ranged from 32.4-ppt (copper - 3 μg/L) to 33.4 ppt (FSW control) and pH ranged from 7.92 (3 μg/L) to 8.11 (FSW control). For zinc treatments, salinity ranged from 33.4 ppt (FSW control) to 27 ppt (Zn 45 μg/L) and pH ranged from 8.10 (FSW control) to 7.95 (Zn 45 μg/L). These salinity and pH levels reflect the natural range of these parameters on the GBR (Kline et al., 2015) and level for high survival for COTS embryos and larvae (Allen et al., 2017; Clements et al., 2022).

Cleavage stage embryos were transferred to vials of nine concentrations of copper (0.1, 0.2, 0.3, 0.5, 1, 1.5, 3, 6, 12 μg/L) and seven concentrations of zinc (2.5, 5, 7.5, 15, 30, 45 μg/L) (n = 5 vials per treatment). Embryo development was scored with respect to the stage reached by control (FSW only) embryos. At 12 h the control embryos were unhatched gastrulae, at 24 h they were hatched gastrulae and at 36 h were late gastrulae. Development data for 24 and 36 hour exposure were only available for zinc because the copper treatments caused 100 % mortality by 24 h.

For the bipinnaria larval stage (six days old) seven concentrations of copper (0.05, 0.1, 0.2, 0.5, 1, 1.5, 3 μg/L) and zinc (0.12, 2.5, 7.5, 15, 25, 45μg/L) were used (n = 5 vials per treatment).). The larvae were scored microscopically for mortality (LC50) and swimming ability (EC50) (motile vs non-motile larvae) at 24- and 48-hours exposure. Impaired swimming was indicated by larvae sinking to the bottom of the vial. This was scored as the percentage of larvae at the bottom of the vial.

For the brachiolaria larval stage (16 days old) eight concentrations of copper (0.1, 0.2, 0.5, 1, 1.5, 3 μg/L) and seven concentrations of zinc (2.5, 7.5, 15, 20, 30, 45 μg/L) were used (n = 5 vials per treatment). The larvae were scored for swimming ability (as above, EC50) and mortality (LC50) at 8 and 32 h after exposure.

2.4. Data analyses

For the embryo tests the percentage stage data for zinc were arcsine square root transformed prior to analysis and analysed as a binomial model in a generalised linear model (GLM with binomial distribution: function glmer, family: binomial), (package lme4, Bates et al., 2015). Due to 100 % mortality in copper, embryo development data were only analysed for zinc. A GLM was used to analyse the percentage survival data for copper (12 h) and zinc (12, 24 and 36 h) to derive the 99 %, 95 %, 90 % and 80 % concentrations for survival as well as the effective concentration for 50 % survival (LC50).

For the larval tests, a GLM was used to analyse the percentage survival data for the bipinnaria and brachiolaria stages at 24- and 48-hour exposure to derive the 99 %, 95 %, 90 % and 80 % concentration for survival as well as the lethal concentration for 50 % survival (LC50). The EC50, the effective concentration, for 50 % of the larvae with impaired swimming was also determined for copper and zinc treatments for bipinnaria and brachiolaria at the 24- and 48-hour time points.

3. Results

3.1. Embryos

Copper had a significant effect on embryo survival with 100 % mortality by 24 h. At 12 h, the LC50 for embryos was at 0.102 μg/L (Table 1, Fig. 1). In the control treatments 100 % of the embryos were hatched gastrulae at 12 h. This embryo stage was also present in the low copper concentration treatments (up to 0.1 μg/L) while at 6 μg/L, 65 % of embryos were arrested at the multicell cleavage stage (Fig. 1). Blastulae were observed in copper treatments ≤ 1.5 μg/L. At 3 μg/L 50 % of the embryos were dead with their cells aggregated to one side. Across all copper treatments there was 100 % embryo mortality at 24 h.

Zinc did not have a significant effect on embryo survival but did impact development (Table 1, Table 2, Fig. 1). At 12 h control embryos were hatched gastrulae while development was delayed in the 7.5 μg/L zinc. Only ~ 50 % of the embryos in this treatment were hatched gastrula (48.18 % ±16 %, n = 5) the rest being delayed as unhatched gastrulae (51.82 % ± 16 %, n = 5) (Fig. 1). These delayed embryos at the 7.5 μg/L concentration hatched by 24 h and most (94.2 % ± 5.3 %) were mid-stage gastrulae at 36 h of exposure. A quarter (25.07 ± 9 %, n = 5) of embryos. In the 15 μg/L treatment ~ 25 % of embryo continued to develop at 36 h. Embryos in the 30+ μg/L treatments were arrested at the early unhatched gastrula stage. At 36-hours, the EC50 for abnormal development was 7.17 μg/L (Table 2). For zinc, there was no significant mortality until 36 h, at which time 62 % (± 6 %, n = 5) of the embryos died in the 60 μg/L treatment.

3.2. Bipinnaria larvae

Copper concentrations had a significant effect on survival of the bipinnaria larvae, with the severity of effects increasing from 24 h to 48 h of exposure (Table 1, Fig. 2). The LC50 for copper at 24 h was 0.67 μg/L (± 0.14 μg/L, n = 5) and at 48 h was 0.54 μg/L (± 0.43 μg/L, n = 5) indicating lower tolerance with time. At 24 h, the ability of larvae to swim was compromised at the lowest dose, 0.2 μg/L with 16.7 % (±10.5 %, n = 5) of the larvae unable to swim. They sank to the bottom of the vial; however, the cilia were still beating and so they were not dead. By 48-hours, most larvae had recovered from their initial sinking response on being placed in copper treatments and regained their swimming ability. Larvae were observed swimming in the water column in copper concentrations up to 0.2 μg/L. At 1 μg/L no individuals were able to swim (Fig. 2). The EC50 for swimming ability was 0.38 μg/L (±0.1 μg/L,

Table 1
Survival of *Acanthaster* sp. bipinnaria and brachiolaria larvae after 12, 24 and 48 hr exposure to copper and zinc treatments with results compared to ANZ Water Quality Guideline 95 % species protection values (1.3 μg/L copper and 15 μg/L for zinc), LC (lethal concentration with 95 %, 90 %, 80 % and 50 % survival) and EC (concentration causing impaired swimming in 50 % of larvae). Standard error in parentheses, $n = 5$. Tolerance at concentrations lower than 95 % species protection guideline values are highlighted in bold.

	Developmental Stage	Timepoint	LC95	LC90	LC80	LC50	EC50
Copper	**Embryo test**	12 h	0	0.022	0.05	0.102	
	Bipinnaria	24 h	0.32 (0.19)	0.45 (0.46)	0.48 (0.37)	0.67 (0.14)	**0.38** (0.1)
		48 h	0.3	0.27 (0.158)	0.42 (0.135)	0.54 (0.43)	**0.35** (0.08)
	Brachiolaria	24 h	0.8 (0.58)	1.31 (0.45)	1.86 (0.36)	**2.82** (0.43)	1.29 (0.2)
		48 h	0.5 (0.2)	0.59 (0.17)	0.68 (0.14)	0.85 (0.11)	0.66 (0.11)
Zinc	**Embryo test**	12 h	>60	>60	>60	>60	18.15 (3.39)
		24 h	>60	>60	>60	>60	11.42 (1.71)
		36 h	>45	>45	>45	45–60	**7.17** (1.21)
	Bipinnaria	24 h		50.85	51.41	52.36	28.89 (0.2)
		48 h	7.75 (5.51)	12.63	17.94	27.009	28.89 (0.2)
	Brachiolaria	24 h	22.7 (0.198)	24.3 (0.14)	26 (0.118)	28.89	**7.18** (0.79)
		48 h	10	11.9	14.6	22	**7.18** (0.79)

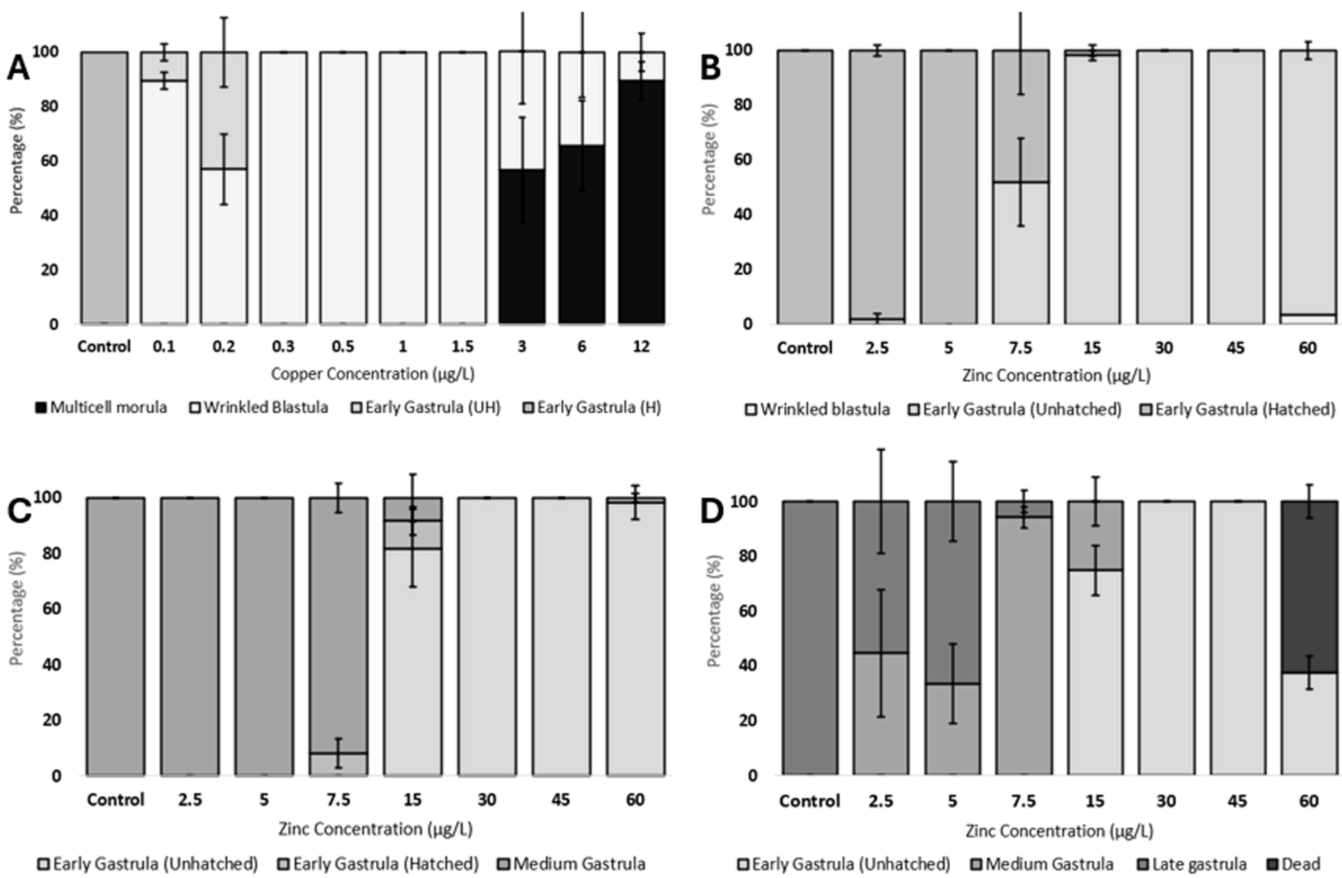

Fig. 1. The impact of copper and zinc on *Acanthaster* sp. embryo development after) 12 h exposure in copper treatments and after b) c) and d) 12, 24 and 36 h exposure to zinc treatments, respectively. (±SE) $n = 6$.

Table 2
Embryo test (GLM analysis), development of *Acanthaster* sp. placed in zinc treatments at the cleavage stage. Development was scored at 12, 24 and 36 h post fertilisation. Significant results are presented in bold; df= degrees of freedom; SE= standard error; $n = 5$.

Developmental Stage	Timepoint	Effect	df	Estimate	SE	Z value	p
Unhatched gastrula	12 h	Intercept		3.9697	1.214	2.269	0.001
		Concentration	38	−0.21867	0.075	−1.883	**0.004**
Hatched gastrula	24 h	Intercept		7.7533	3.2304	1.400	0.0164
		Concentration	38	−0.6791	0.2771	−1.451	**0.0143**
Late gastrula	36 h	Intercept		1.6852	1.6852	2.440	0.0147
		Concentration	38	0.2615	0.2615	−2.194	**0.0282**

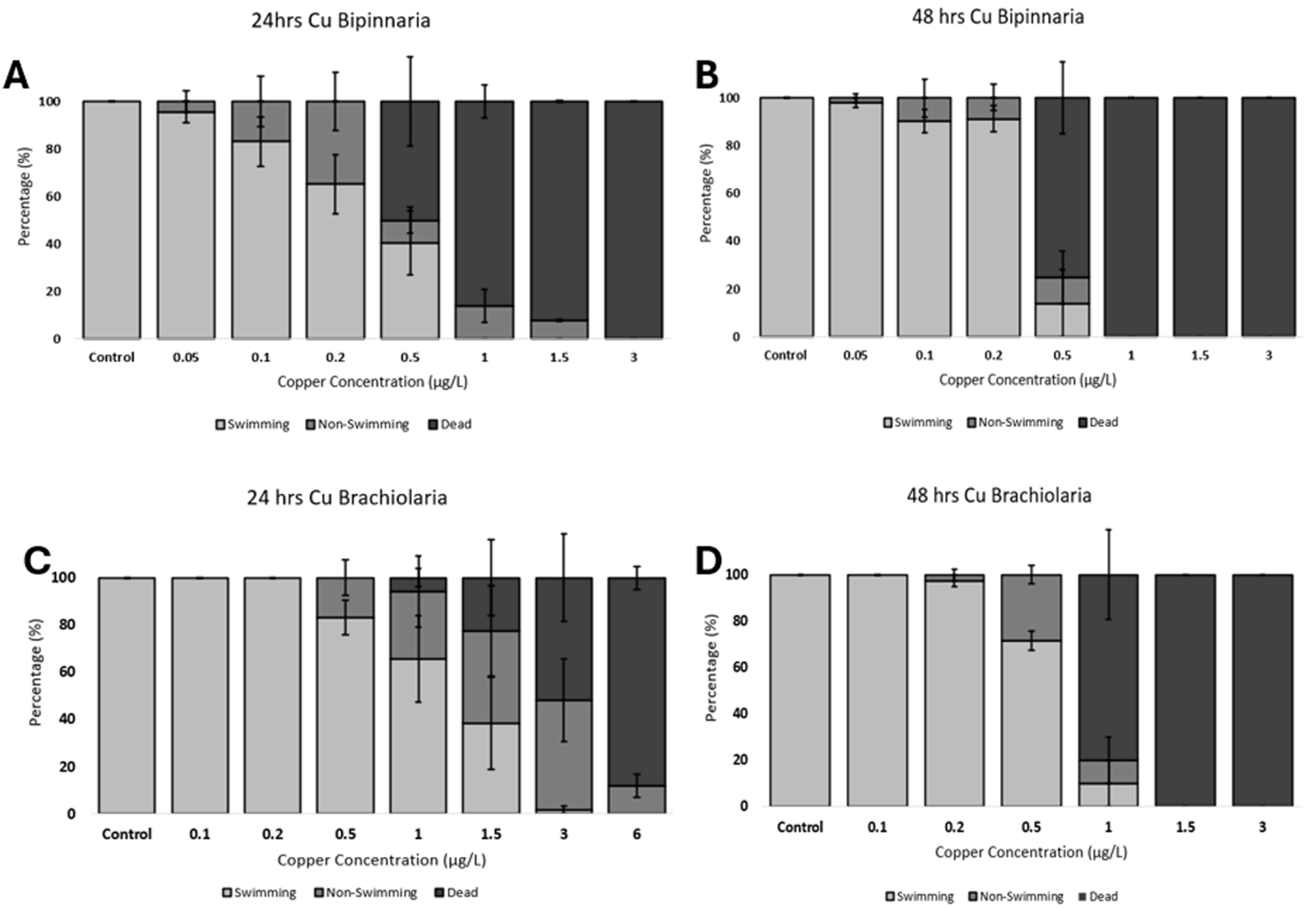

Fig. 2. The effects of copper on *Acanthaster* sp. swimming behaviour and survival of larvae at a) bipinnaria larvae after stage 24 h exposure b) bipinnaria larvae after 48 h exposure c) the brachiolaria larvae after 24 h exposure d) brachiolaria larvae after 48 h exposure (±SE) ($n = 5$).

$n = 5$) at 24 h and 0.35 μg/L (±0.08 μg/L, $n = 5$) at 48 h.

Zinc did not affect survival at 24 h but had an effect at 48 h of exposure (Table 1, Fig. 3). The LC50 for bipinnaria exposed to zinc decreased from 52.36 μg/L (0.96 μg/L, $n = 5$) at 24 h to 27.01 μg/L (1.07 μg/L, $n = 5$) at 48 h exposure (Table 1, Fig. 3). Zinc treatments negatively impacted swimming ability at both 24 h and 48 h of exposure (Table 2). The EC50 for swimming at 24 and 48 h was 28.89 μg/L (±0.2 μg/L, $n = 5$). Swimming ability was maintained during the 48-hour experimental period in all larvae in the 2.5 μg/L treatment and in 94.76 % of individuals in the 7.5 μg/L treatment (Fig. 3). At 24 h swimming ability was completely (100 %) or mostly (90 %) lost in larvae in the 45 μg/L and 25 μg/L treatments, respectively. By 48 h 90 % of the larvae in the 25 μg/L treatment were not swimming.

3.3. *Brachiolaria larvae*

Copper had a significant effect on brachiolaria survival (Fig. 2) with an LC50 decreasing from 2.82 μg/L (±0.34 μg/L, $n = 5$) at 24 h to 0.85 μg/L (±0.11 μg/L, $n = 5$) at 48 h exposure (Table 2). With respect to swimming ability, a significant impact of copper was observed at both 24 h and 48 h of exposure (Table 1, Fig. 2). After 24 h, no larvae were able to swim in the 3 μg/L zinc treatment. The EC50 for copper decreased from 1.29 μg/L (± 0.2 μg /L) to 0.66 μg/L (± 0.11 μg /L) between 24 and 48-hour exposure periods (Table 2). By 48 h in the 1.5 μg/L copper treatment there was 100 % mortality (Fig. 2).

Zinc had a significant effect on survival and swimming in the brachiolaria, with mortality increasing from 24 h to 48 h exposure (Table 1, Fig. 3). The LC50 at 24 and 48 h was 28.89 μg/L (± 0.82 μg /L) and 22 μg/L (± 1.1 μg/L), respectively. Mortality was 100 % in the 45 μg/L treatment. Larval swimming ability was maintained during the 48-hour experiment in the 2.5 μg/L treatment. At the 24-hours, larvae were swimming in the 15 μg/L treatment, however a few of these (12 %) recovered their swimming ability at 48 h. In the 7.5 μg/L treatment, 40 % of individuals were swimming at both the 24 and 48 h timepoints, with EC50 being 7.18 ug/L (±0.79 μg/L, $n = 5$) (Table 1, Fig. 3).

4. Discussion

Survival of the developmental stages of *Acanthaster* sp. significantly decreased in response to exposure to copper and zinc with embryos being the most sensitive. Elevated concentrations of copper and zinc retarded or inhibited development and impaired larval swimming and survival. The bipinnaria larvae lost their ability to swim at 0.38 μg/L Cu. This is lower than the guideline concentrations used for 95 % species protection. The bipinnaria were less sensitive to zinc exhibiting normal development and swimming at 28.9 μg/L, levels above the 95 % species protection. For the brachiolaria larvae, swimming ability was lost at 0.66 μg/L for copper and 7.2 μg/L for zinc. These results indicate that levels used for 99 % species protection (ANZG, 2018) are more appropriate at this developmental stage. As predicted, embryonic development was delayed or arrested at the high concentrations of copper (> 0.32 μg/L) with likely mortality at the upper concentrations as these embryos did not progress by the second time point. For zinc, arrested development in embryos was observed by 36 h at 7.17 μg/L. At lower concentrations of both metals some larvae regained their swimming ability after initial exposure.

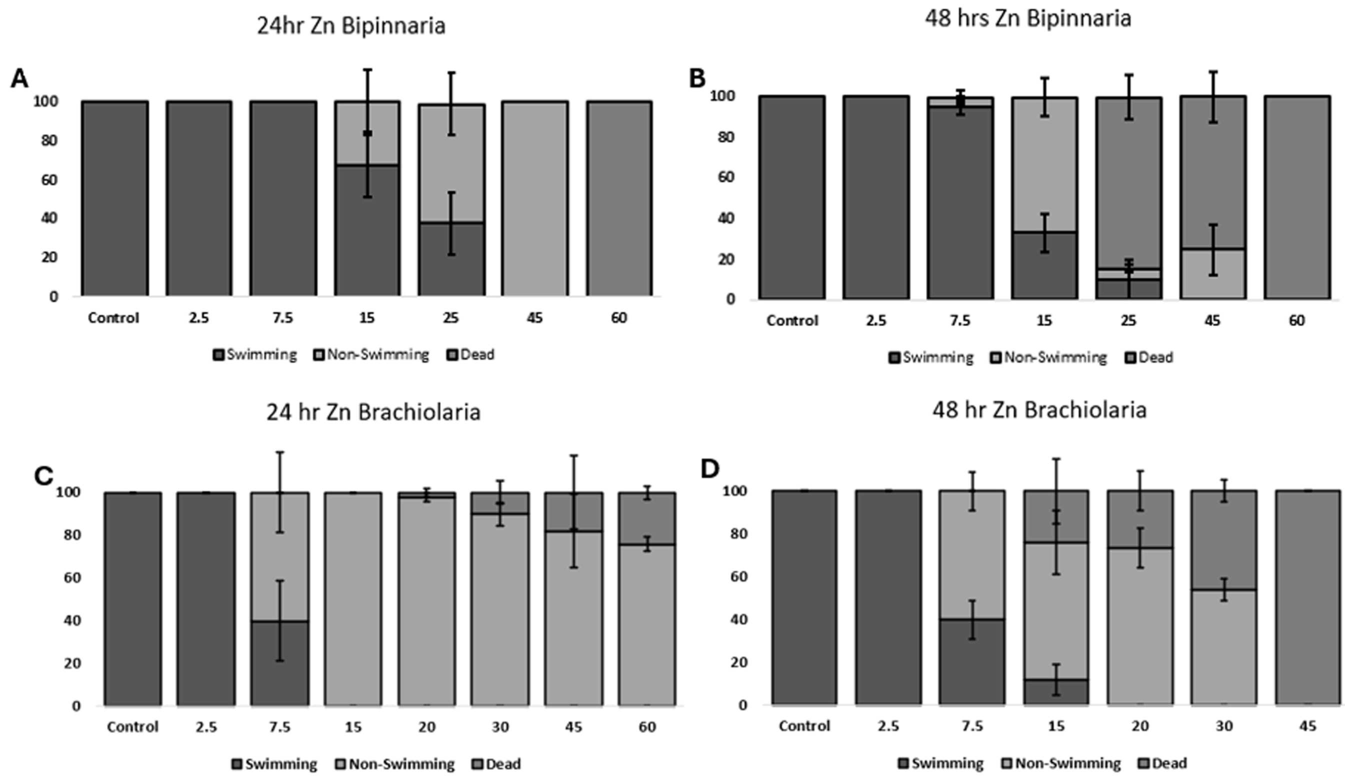

Fig. 3. The effects of copper treatments on *Acanthaster* sp. swimming behaviour and survival on larvae at a) the bipinnaria stage 24 h exposure b) the bipinnaria stage at 48 h exposure c) the brachiolaria stage at 24 h exposure and d) and at the brachiolaria stage at 48 h exposure at 24 and 48 h after exposure to copper treatments (±SE) ($n = 5$).

From the very few data published for waters of the GBR (Sup Table 1), it appears that copper has been recorded in concentrations that would be harmful for COTS development. Background levels of copper recorded on mid and outer shelf reefs on the GBR (Lizard Island, 0.13 µg/L and Heron Island, 0.13 µg/L, respectively) (Denton and Burdon-jone, 1986) would be deleterious to *Acanthaster* embryos but not larvae. Based on the results of the present study, for embryos concentrations of copper recorded in nature would cause developmental delay. However, as COTS embryos hatch in the benthos and likely remain in the proximity of the sea floor, they are unlikely to have an exposure pathway to encounter copper pollution in early development. Concentrations of copper recorded in GBR waters would be unlikely to impact the swimming ability and survival of COTS larvae (i.e., EC50 – bipinnaria: 0.32, brachiolaria: 0.38 µg/L). Zinc levels recorded for offshore reefs (Lizard Island 0.08 µg/L, Heron Island 0.17 µg/L) would also be unlikely to impact COTS embryo development and survival or the swimming ability of COTS larvae.

Field surveys based on e-DNA studies, indicate that the larvae of COTS are broadly distributed in GBR waters over a vast spatial scale including in regions occasionally impacted by runoff plumes (Uthicke et al., 2015; Kroon et al., 2023). If the larvae are dispersed shoreward in currents, they may encounter lethal levels of copper, and this may contribute to the general absence of COTS on inshore reefs. In association with shipping and leaching of antifoul hull coverings, ports would be a source of copper. For instance, near Cape Grafton adjacent to the mid GBR, copper concentrations of 3.07 µg/L have been measured in the water (Ports North, 2015). Waters in the proximity of ports with high zinc concentrations such as Trinity Bay (13.6 µg/L) and Cape Grafton (14.3 µg/L) (Sup Table 1) (Ports North, 2015) could impact larval swimming ability but not survival of COTS larvae. Along with the runoff driven sediment loads and turbidity (Kroon et al., 2020) the presence of metal pollution in near shore waters may contribute to the absence of COTS on inshore reefs (Waterhouse et al., 2017).

With respect to copper the LC50 for the larval stages of COTS was 0.54 µg/L for bipinnaria and 0.85 µg/L for brachiolaria. The EC50 for swimming ability was lower at 0.35 µg/L for bipinnaria and 0.66 µg/L for brachiolaria stages after 48 h of exposure. While the larvae were not dead, they were unable to swim and this would compromise their survival. That said, cessation of swimming may also be a mechanism to sink below unfavourable surface plumes (Clements et al., 2022) where toxicants are likely to be more prevalent. In a salinity study the larvae of COTS sank at 25–30 ppt (Clements et al., 2022), a response seen in the larvae of several echinoderms (Metaxas and Young, 1998). Cessation of swimming where echinoderm larvae sink but remain alive with cilia beat evident has been observed in response to elevated copper (100 µg/L) in the larvae of the sea urchin, *Strongylocentrotus intermedius* (Yaroslavtseva and Sergeeva, 2008). Thus, as found for sea urchin larvae, the cilia were still beating in the COTS larvae that sank in response to copper and zinc, but clearly the mechanism of the ciliary beat was altered as they did not perform the normal swimming function.

As copper is routinely used as a reference toxicant for regulatory purposes to assess the toxicity of marine waters (e.g., Doyle et al., 2003), the data generated here on the response of the embryos and larvae of COTS to this metal can be placed in an international ecotoxicology context especially with respect to data available for echinoderms (Sup. Table 2). Overall, in comparison to other echinoderms, the embryos and larvae of COTS showed higher sensitivity to copper while our results for zinc were similar to that determined by previous studies (Sup. Table 2). There is however a general paucity of ecotoxicology data for sea star embryos and larvae with which to compare our data (Sup. Table 2) and it would be expected that sea stars and sea urchins differ in their sensitivity to the metals investigated here.

In comparison with studies of other echinoderms, the developmental stages of COTS are more sensitive to copper and zinc (Sup. Table 2). For copper the LC50 concentrations for COTS development are 5-to-200-fold lower than that determined for tropical and temperature sea cucumber species (Heslinga 1976; Kobayashi 1980; Ramachandran et al. 1997). For zinc LC50 concentrations for COTS development are 5-fold lower than that for *Lytechinus variegatus* (Nipper et al., 1993) and similar to the LC50 determined for *Peronella japonica* and *Strongylocentrotus purpuratus* (Kobayashi, 1980; Cunningham et al., 2020). A potential reason for the comparative sensitivity of COTS particularly to copper may be associated with the pristine habitat in which the developmental stages normally occur. For the gastropod *Crepuidula fornicata*, juveniles from polluted sites were over two-fold more tolerant of copper (LC50: 301 μg/L at reference site vs LC50: 636 μg/L at polluted site) than juveniles from the reference pristine site (Untersee and Pechenik, 2007). These authors suggest that the gastropod juveniles exhibited that local adaptation to pollutants.

Interestingly, coral development is far less sensitive to copper and zinc than that of COTS. Studies of development of corals from the GBR and Ningaloo Reefs show that coral development to metamorphosis tolerates copper concentrations in the range of 14 μg/L – 100 μg/L (Esquivel, 1986; Howard et al., 1986; Reichelt-Brushett and Harrison, 1999, 2000; Negri and Heyward, 2001; Reichelt-Brushett and Hudspith, 2016). These are 15-to-30-fold higher concentrations than determined here for *Acanthaster* development. Similarly, the tolerance of development in the coral *Goniastrea* aspera to zinc (Reichelt-Brushett and Harrison, 1999) is at concentrations up to eight-fold higher than that determined for COTS embryos and larvae.

Our results for the thresholds, EC50 for lethal effects of Cu (0.32 – 0.38 μg/L) and Zn (22- >60 μg/L) for development of COTS, indicate that this is a sensitive species, based on the concentrations established for 95 % protection of marine life (ANZG 2018). Using these current guidelines for 95 % species protection for copper (1.3 μg/L) and zinc (15 μg/L) (ANZG 2018), *Acanthaster* embryos would not survive or advance to later stages. For copper the LC50 for *Acanthaster* embryos and bipinnaria larvae were 0.102 and 0.54 μg/L, respectively, lower than the 1.3 μg/L used for 95 % species protection. Although later stage larvae, the brachiolaria were more tolerant with the mortality thresholds, LC50 for deleterious effects of copper after 24 h (2.048 μg/L) had values above the 95 % species protection levels (ANZG) but below the guideline levels at 48 h (0.85 μg/L). In contrast, LC50 values were above the 95 % species protection levels (ANZG) for zinc.

With respect to the sub-lethal parameter assessed for COTS larvae, swimming ability, copper is deleterious at the 95 % species protection levels (ANZG, 2018) for the bipinnaria larvae, but this is not the case for zinc. The EC50 was 0.35 μg/L in copper and at a concentration of 27 μg/L zinc after 48 h of exposure. For brachiolaria, 50 % of the larvae tolerated 0.66 μg/L copper. Zinc exposure had a greater effect on brachiolaria than bipinnaria, with the EC50 value being at 7.2 μg/L and 28.89 μg/L, respectively. For brachiolaria, this aligns with the 99 % species protection value of 7 μg/L. The swimming response appears to be a good metric to assess toxic concentrations in the two metals tested.

Our findings indicate that, for copper, the guideline concentrations (ANZG, 2018) would not protect COTS embryos and larvae. However, the zinc concentration guideline concentrations would protect these developmental stages. For zinc, the LC50 is higher than the 95 % species protection limit (15 μg/L) for all stages and timepoints. Thus, for all developmental stages tested for zinc the ANZG would be protective. However, despite surviving at the 95 % species protection limit, the EC50 for swimming was 7 μg/L at the brachiolaria stage, lower than the 95 % species protection guideline value. This shows the importance of considering sublethal traits. With respect to larval swimming trait, the 99 % species protection value of 7 μg/L would be more suitable. This level of species protection is seldom used when assessing water quality as the ANZG recommends the '*high reliability trigger value of 15 μg/L*'. The level for swimming impairment is nearly double the current trigger value, a result that has implications for a great diversity of species that have a similar larval stage to COTS, with similar ciliary-driven swimming, including other echinoderms and molluscs.

The current study, like other investigations used to derive the ANZG (2018), are based on single-chemical ecotoxicological experiments performed under optimal conditions and do not account for additional stressors that can affect the toxicity of contaminants (Noyes et al., 2009; Zhou et al., 2014; Negri et al., 2020; Castro-Sanguino et al., 2021). Echinoderm embryonic stages are generally more tolerant to stress than larval stages (Hamdoun and Epel, 2007; Allen et al., 2017; Balogh and Byrne, 2020), but this depends to on the stressor (Byrne, 2011). As we show here, COTS larvae were more tolerant to copper and zinc than embryos, highlighting the importance of multiple stressor studies. Reduced water quality in inshore areas of the GBR Marine Park is a result of the increased loads of fine sediment, nutrients and pesticides discharged from land use changes in the GBR catchment (Kroon et al., 2020). Thus, a single water quality variable may be of limited use to identify the impacts of regional population effects with respect to global stressor impacts (Holan et al., 2019; Castro-Sanguino et al., 2021). In particular, heatwaves with temperature spikes well above historic means are devastating for coral reef and other tropical ecosystems (Hughes et al., 2017; Mentzel et al., 2024; Huang et al. 2024; Byrne et al., 2025). It is crucial to consider the synergistic impacts of habitat warming on the impact of contaminants and implications for water quality guidelines.

Many studies have shown that increased temperature increases the sensitivity of marine ectotherms to contaminants (Noyes et al., 2009; Zhou et al., 2014). Results indicate that the negative effects of metal exposure (Gadolinium) on *Paracentrotus lividus* larval development will be mitigated by near-future ocean warming, up to a thermotolerance threshold when negative synergistic effects were evident (Martino et al., 2021). A recent modelling study predicts that temperature increase at the level that causes coral bleaching (1–2 °C) should be considered through application of multistressor risk assessments (Negri et al., 2020). Empirically understanding the effects of temperature on the impacts of contaminants on planktonic larvae and assessment of marine water quality guidelines is essential for environmental protection measures.

This study highlights the importance of understanding the response of tropical marine invertebrates to anthropogenic pollutants in deriving and applying marine water quality guidelines. For the region in focus, the GBR and tropical waters elsewhere, our results with *Acanthaster* sp will inform the development of more targeted, relevant marine water quality guidelines. Our data for COTS development may also be used to indicate sensitivities of co-occurring animals and life stages living in the plankton in GBR waters. The current guidelines for copper and zinc were informed by an analysis of available data on adult and larval of marine invertebrate species from six taxonomic groups, largely temperate species, with no data for echinoderms. As the guidelines encourage the conduct of site-specific investigations (ANZG, 2018), this study compliments regional studies as a consideration when deriving GBR water quality management guidelines.

CRediT authorship contribution statement

Regina Balogh: Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Byrne:** Writing – review & editing, Supervision, Resources, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2025.107357.

Data availability

Data will be made available on request.

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