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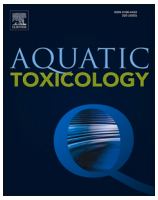

Cnidarian models for toxicology

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ABSTRACT

Coral reefs and tropical habitats are threatened worldwide by global warming and pollution stress. The purpose of these studies was to evaluate potential strategies for using jellyfish for toxicological assessments and as potential toxicological models for corals and other Cnidarians. Laboratory studies were conducted with jellyfish and three species of corals that were exposed to copper; and studies with corals exposed to pyrene and elevated temperatures were conducted. Observational (pulsation rate in jellyfish and bleaching in corals) as well as cellular biomarker responses (glutathione (GSH), lysosomal destabilization, and tissue Cu in jellyfish; GSH in corals) were assessed. Jellyfish pulsation rate, lysosomal destabilization, and tissue Cu levels were significantly correlated. Likewise, GSH levels were significantly correlated with tissue Cu, lysosomal destabilization and pulsation rates. Jellyfish tended to be more sensitive than corals to Cu exposures. Studies were conducted with adults and larvae of brain corals and other species from Curaçao to determine the baseline glutathione levels. Glutathione levels of these Cnidarians were much lower than those of more traditional bioindicators such as mussels or oysters. Glutathione levels of adult jellyfish were lower than adult coral levels. The GSH levels of early life history stages of corals (especially larvae) were lower than adult levels, potentially indicating that these stages could be more sensitive than adults. The GSH levels of the younger coral stages were similar to the GSH levels of jellyfish adults. Species-specific differences in the sensitivity of corals to the different pollutants were observed. This work was facilitated by partnerships with Discovery Place Science (a public science exploration center), CARMABI (Caribbean Research and Management of Biodiversity), and SECORE International which are actively engaged in the culture of Cnidarians and marine educational programs.

1. Introduction

Marine ecosystems, especially coastal tropical habitats such as coral reefs, are threatened worldwide by global warming and pollution (Hughes et al., 2003; Mitchelmore et al., 2021; Woesik et al., 2022). Increases in temperatures of only a few degrees centigrade can have devastating consequences, especially for sensitive species. Coastal development and ecotourism contribute pollutants from sewage, street run-off, and personal care products, including metals, pesticides, and sunscreens which can also exacerbate temperature impacts (Negri and Hoogenboom, 2011; Richmond et al., 2018). While restoration interventions are essential for facilitating the re-establishment of damaged reef systems (Banaszak et al., 2024; Chamberland et al., 2015, 2017a; Donner et al., 2005; Knowlton et al., 2021; Vardi et al., 2021), toxicological assessments may further provide a better undersanding of pollutant risks and effects. More traditional bioassay models such as bivalves, crustaceans, and fish may not adequately reflect potential impacts on Cnidarian species - corals, anemones, and jellyfish. Extensive sampling of corals for toxicological studies must be limited and in some cases is prohibited given declining populations of many species (Gutierrez et al., 2024). To assess potential pollutant risks and effects, toxicological models that are based on coral and other Cnidarian responses are emerging. While more laboratories are developing substantial expertise in the culture and breeding of corals (Banaszak et al., 2024), good surrogate species that are readily cultured, such as jellyfish and anemones, are important bioassay organisms that can be used for

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studies related to protecting coral and other Cnidarian-rich ecosystems (Rosner et al., 2024). A variety of valuable behavioral and cellular responses to pollutants are being investigated, including oxidative stress, metabolomic responses related to mitochondrial dysfunction, and development of cell culture methods (Ventura et al., 2018; Ianna et al., 2020; Stien et al., 2020; Roepke et al., 2022; Thorel et al., 2022). Therefore, the purposes of these studies were to investigate behavioral and cellular responses of moon jellyfish (*Aurelia aurita*), a valuable bioindicator species for assessing potential impacts of anthropogenic stressors, and to consider their relative sensitivity to corals and coral developmental stages.

Short-term laboratory studies were conducted with jellyfish *A. aurita* and three Indo-Pacific coral species that were cultured at *Discovery Place Science* (Charlotte, NC USA) and exposed to copper (Cu). Corals were also exposed to an elevated temperature treatment and pyrene. Observational endpoints (pulsation rate in jellyfish and bleaching in corals) were used to evaluate the potential value of non/less-destructive endpoints. Biomarker responses (lysosomal destabilization, glutathione, and tissue Cu) were then determined in jellyfish. Baseline levels of glutathione (GSH) were also determined in larvae and adults of three Caribbean coral species, that were provided by scientists at the *Caribbean Research and Management of Biodiversity* (CARMABI, Willemstad, Curaçao).

2. Methods

2.1. Jellyfish exposure studies

Laboratory exposures were conducted with jellyfish (*A. aurita)* medusae cultured by Discovery Place Inc., a science education museum in Charlotte, NC USA. For each experiment (four total), a range of Cu solutions were prepared from a 500 ppm stock solution of cupric chloride. All Cu exposures were prepared in Instant Ocean saltwater, 25-30 ppt salinity and 7.95-8.05 pH. Jellyfish were exposed to 0 (control), 5, 10, 25 and 50 µg/L Cu treatments for 48 hours. For each experiment, four to five jellyfish were placed in 2 gallon buckets with 7 L of control or Cu treatments (2-3 replicate buckets per treatment). Treatment water was changed daily. During the 48-hour exposures, jellyfish were fed brine shrimp nauplii (*Artemia salina*) and exposures were performed at room temperature (18-20°C) with constant gentle aeration. Salinity and pH were monitored and recorded daily for each bucket. At the end of 48 hours, animals were dissected and bell tissues were collected for biomarker and tissue Cu analyses. Freshly dissected bell tissues were used for lysosomal destabilization assays, and bell tissue samples were frozen for total GSH and tissue Cu analyses.

Behavioral observation endpoints were assessed visually and recorded for each individual jellyfish at the beginning, during (after 24 hours), and at the end (after 48 hours) of each exposure experiment. Pulsation rate (pulses/minute) were recorded for 1 minute intervals. Feeding behavior (whether or not individuals actively ingested food) was noted. Water column position (if they continued swimming in the water column or tended to stay at the bottom of each bucket) was recorded.

A neutral red lysosomal destabilization assay was used to assess the integrity of lysosomal membranes and cellular toxicity (Ringwood et al., 2005). Freshly dissected bell tissue samples of each jellyfish (approximately 150-250 mg), were processed into primary cell preparations with Ca/Mg-free-saline (CMFS) and trypsin, sheared to break apart clumps of cells and release hemocytes, and filtered through 41 µm nylon screens. Cells were rinsed and re-suspended in CMFS and a stock solution of neutral red (NR) (0.04 mg/mL) was added at a 1:1 ratio of NR to cell preparation volume. After a 60-minute incubation period, cells were scored as either stable (NR contained within the lysosomes) or destabilized (NR diffusing into the cytoplasm from damaged lysosomes) at 400× magnification, with at least 50 cells scored from each individual jellyfish preparation, and summarized as % destabilized lysosomes per individual. Examples of stable and destabilized jellyfish hemocytes are provided in Supplemental Fig. 1.

Glutathione (total GSH) levels in bell tissues were determined using the DTNB-GSSG Reductase Recycling assay (Ringwood et al., 1999). Tissues were weighed and homogenized in 4 volumes of 5% sulfosalicylic acid (SSA) and centrifuged (13000 g at 4°C for 5 minutes). Subsamples of the supernatant (100 µL) were further diluted with 200 µl of SSA. Standards of known glutathione concentrations were prepared (1.5-200 µM) from a 1 mM GSH stock solution (Sigma-Aldrich, MO). Samples and standards (30 µL, 3 replicates per sample) were added to 96-well plates with 150 µl of nicotinamide adenine dinucleotide phosphate (NADPH, 0.238 mg/ml), 40 µL of 5,5'-dithiobis (2-nitrobenzoic acid (DTNB 10 mM, Sigma-Aldrich), 23 µL of water. Glutathione reductase (7 µL, 50 U/mL, Sigma-Aldrich) was added to each well and a kinetic analysis (20 second reads for 2 minutes) was performed using a MultiSkan Go 3.2 UV/Vis spectrophotometer (Thermo Fisher Scientific) at 412 nm. Results were expressed as nmol GSH/g wet tissue.

Copper tissue concentrations were analyzed using atomic absorption spectrometry. Samples of bell tissue were lyophilized using a VirTis benchtop K Lyophilizer. Each sample was then weighed (dry tissue weight), homogenized and microwave digested in ultra pure nitric acid. Fully digested samples were then diluted with ultra pure water. Samples, standards and oyster standard reference tissues were then analyzed using a Perkin Elmer AAnalyst 200 (Waltham, MA, USA) atomic absorption spectrometer using furnace mode and Deuterium background correction. Results were expressed as µg Cu/g of dry tissue weight. The standard oyster tissue concentrations for Cu ranged between 95 101% of expected values.

2.2. Coral laboratory exposure studies

Three species of corals, *Montipora capricornis, Caulastrea furcata,* and *Acropora divaricata* were provided from the aquarium and culture facilities at Discovery Place. Fragments of each species were exposed for 48 hours to elevated temperatures (increased gradually from 22°C to 26°C over 24 hours), 2 concentrations of Cu (50 and 200 µg/L), and 2 concentrations of pyrene (10 and 100 µg/L). Three replicate fragments of each species were exposed to control or experimental treatments in separate 1 L beakers of Instant Ocean saltwater, 30 ppt salinity, and 7.95-8.05 pH. Visual observations of the corals were conducted to identify signs of bleaching, the primary endpoint, but the excess production of mucus was also recorded as a stress response.

2.3. Glutathione levels of field-collected corals, Eggs, Embryos, and Larvae

Coral samples were collected by CARMABI staff from coastal sites in Willemstad, Curaçao for the determination of baseline GSH levels. Small heads or fragments (approximately 2–4 cm diameter) of three species, *Diploria labyrinthiformis, Tubastraea coccinea*, and *Agaricia humilis*, were wrapped in aluminum foil, frozen on dry ice, and transported back to UNC-Charlotte for processing. We were also able to obtain eggs, embryos, and larvae for *D. labyrinthiformis* that were collected and cultured by CARMABI and SECORE staff as part of their coral breeding, research, and restoration programs (Chamberland et al., 2017b; Marhaver et al., 2015). The staff have identified when this broadcast spawning species with external fertilization is likely to spawn, and divers collect gametes from spawning coral heads, which are then taken back to the laboratory where they are cultured (Chamberland et al., 2017b). Eggs from multiple coral heads were combined, fertilized and aliquoted to 10 separate plastic culture containers. Multiple samples of unfertilized eggs (n=4, each comprised of approximately 500 eggs) were taken from the egg mixture. Samples of embryos (4 hours post-fertilization) and planula larvae (20 hours post-fertilization) were taken from 4 random culture containers (n=4, each comprised of approximately 200 embryos or larvae). Eggs, embryos, and larvae were frozen for GSH analyses.

Tubastraea coccinea and *Agaricia humilis* are brooding species with internal fertilization that releases fully developped larvae. Adult colonies were collected from the reef and held in a flow through seawater system; larvae that were released were collected daily from overflow mesh containers.

Fragments of frozen samples (approximately 1 cm^2) of adult corals (from 4 – 6 different coral heads for each species) were suspended in 5% SSA and vortexed vigorously for at least 30 seconds. Small coral fragments or scraped samples included mostly soft tissues and minimal amount of calcified skeleton. During the vortexing step the calcified parts served to help macerate the tissues and release the zooxanthellae. The tissues were then gently centrifuged so that most of the hard calcified pieces and zooxanthellae (which tended to remain intact) as well as nematocyts settled to the bottom. The supernatants were collected, and processed for GSH as described in Section 2.1 above. The final tissue pellets were drained to remove excess water, and the pellets were then weighed for determination of tissue wet weight (pellet weights ranged from 10–30 mg).

Samples of eggs, embryos, and planula larvae were homogenized with a glass homogenizer in 5% SSA. These developmental stages do not contain any calcified parts or zooxanthellae, The homogenates were processed for GSH as described in Section 2.1 above. The final tissue pellets were drained to remove excess water, and the pellets were then weighed for determination of tissue wet weight (16–54 mg for egg and embryo samples, and 10-30 mg for larval samples).

2.4. Statistical analyses

Data were analyzed using Sigma Stat 2.0 and Graphpad Prism 6. For the jellyfish studies, the data summaries were based on at least 20 jellyfish for each treatment over 4 experiments. Analysis of Variance (ANOVA) was used to compare treatments and post-hoc pairwise comparisons (Student-Neuman Kuels, Tukey, or Dunn's) were further used to determine significant differences between treatment groups and controls. Regression analyses were used to identify relationships between tissue Cu accumulation and lysosomal destabilization, tissue Cu accumulation and pulsation rate, and lysosomal destabilization and pulsation rate. Additionally, regression analyses and linear extrapolation were used to determine dose-dependent responses and EC50 estimates for lysosomal stabilization and pulsation rates. For the coral studies, ANOVA was used to identify species-specific differences in GSH levels of adults, and GSH differences between life history stages of brain corals.

3. Results

3.1. Behavioral responses of jellyfish (Aurelia aurita) exposed to Cu

Routine pulsation, active swimming, and active feeding are regarded as normal jellyfish behavior. While no changes in the pulsation rates of jellyfish were observed for 5 and 10 μg/L Cu, a significant decrease in the pulsation rates of jellyfish was observed at 25 μg/L Cu after 24 and 48 hours of exposure (Table 1). After 48 hours, jellyfish exposed to 25 μg/L Cu also exhibited other alterations in swimming behavior, spent more time on the bottom of the containers, and tended not to feed. There was 100% mortality in jellyfish exposed to 50μg/L Cu after 24 hours. The 48 hour EC50 estimate for the effective concentration at which pulsation rates were reduced by 50% was approximately 20 μg/L Cu.

3.2. Biomarker responses and tissue Cu levels of jellyfish (Aurelia aurita) exposed to Cu

Dose-dependent increases in lysosomal destabilization rates were observed with increasing Cu exposure concentrations (Fig. 1) as well as increases in tissue Cu levels. There was a significant relationship between lysosomal destabilization and tissue Cu levels (Fig. 2a), and also between tissue Cu and pulsation rates (Fig. 2b), as well as between lysosomal destabilization and pulsation rates (Fig. 2c). A 48 hour EC50 based on a 50% increase in lysosomal destabilization over the controls was estimated as 11 μg/L Cu.

There was a significant tendency for increasing GSH levels with increasing tissue Cu concentrations (Fig. 3a), and there were also significant correlations between GSH and lysosomal destabilization (Fig. 3b), as well as GSH and pulsation rates (Fig. 3c). The GSH levels of

Table 1
Aurelia aurita, observational studies for Cu exposure studies.
Pulsation rate and swimming and feeding behavior were recorded. √ Indicates swimming and feeding normally; X indicates abnormal swimming or little to no feeding

Treatment	Observation Time	Pulsation Rate (pulses / min)	Swimming	Feeding
Control	Time 0	31.5 ± 1.2	✓	✓
	24 hr	32.8 ± 3.0	✓	✓
	48 hr	27.6 ± 6.8	✓	✓
5 ppb Cu	Time 0	36.5 ± 0.8	✓	✓
	24 hr	31.5 ± 4.7	✓	✓
	48 hr	29.6 ± 3.5	✓	✓
10 ppb Cu	Time 0	31.6 ± 1.1	✓	✓
	24 hr	26.5 ± 4.0	✓	✓
	48 hr	26.1 ± 4.4	✓	✓
25 ppb Cu	Time 0	32.8 ± 1.5	✓	✓
	24 hr	16.5 ± 2.5	✓	✓
	48 hr	9.6 ± 3.9	X	X
50 ppb Cu	Time 0	32.0 ± 1.7	✓	✓
	24 hr	0	X	X
	48 hr	0	X	X

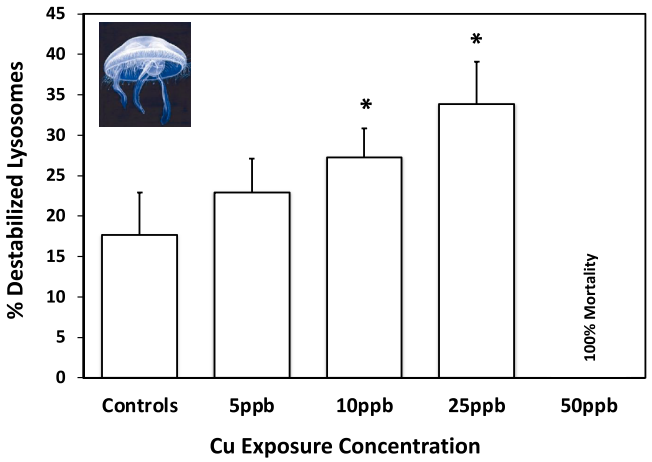

Fig. 1. *Aurelia aurita*, lysosomal destabilization after 48 hour Cu exposures. Data are means + standard deviation of 4 different experiments, each with 4-5 individual jellyfish for each treatment. Asterisks (*) indicate significant difference from controls ($p<0.05$).

control jellyfish tended to range from approximately 10-25 nmol GSH/g wet weight.

3.3. Coral laboratory exposure studies

There were some notable species specific and stressor specific differences for the three species used for these studies (Table 2). *Acropora divaricata* exhibited severe bleaching when exposed to temperature stress and pyrene at 10 and 100 µg/L. While no bleaching was observed with this species exposed to 50 µg/L Cu for 48 hours, moderate bleaching was observed at 200 µg/L Cu. Moderate bleaching was observed in *Montipora capricornis* exposed to both concentrations of pyrene, but no bleaching was observed in the elevated temperature treatment. When *M. capricornis* was exposed to Cu, moderate bleaching was observed with 50 g/L and severe bleaching was observed at 200 µg/L (illustrated in Supplemental Fig. 2 photographs). *Caulastrea furcata* was the least sensitive of the three species; bleaching was only observed at the higher concentration of pyrene, but no bleaching was observed for Cu or elevated temperatures. Excess mucous production was observed with *C. furcata* exposed to Cu, indicating a moderate stress response.

3.4. Glutathione levels of field-collected corals, eggs, and larvae

The GSH levels of the three adult Caribbean corals, *D. labyrinthiformis, T. coccinea*, and *A. humilis* were similar, ranging from 300 – 500 nmol/g (Fig. 4). While egg GSH levels of *D. labryrinthiformis* were similar to adult levels, GSH levels declined over the course of development. Glutathione levels of planula larvae were significantly lower than adults, eggs, and embryos; larval levels were approximately 57 nmol/g (Fig. 4). Due to limited numbers of *T. coccinea* and *A. humilis* larvae, only one pooled sample was analyzed for these two species, which had very low levels of approximately 10 and 3 nmol/g GSH, respectively.

4. Discussion

These studies provide novel results regarding the behavioral and cellular responses of a ubiquitous jellyfish (*A. aurita*) to Cu exposures. Lysosomal destabilization is widely recognized as a valuable sensitive biomarker of pollutant and toxin exposure and effects in a variety of more traditional bioindicator species such as bivalves and fish (Regoli, 1992; Moore, 2006; Moore et al., 2006; Farrington et al., 2016). These are the first studies to demonstrate that lysosomal destabilization is also a valuable sensitive biomarker in jellyfish. The lysosomal destabilization

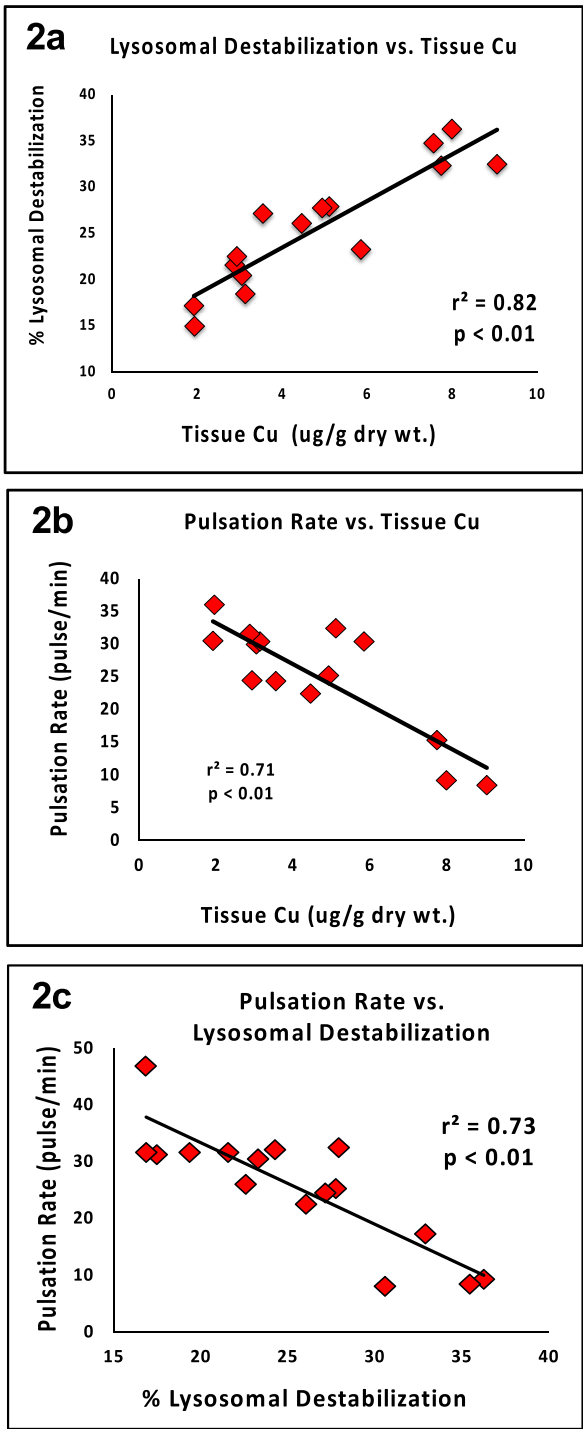

Fig. 2. *Aurelia aurita*, relationships between lysosomal destabilization and tissue Cu (a), pulsation rate and tissue Cu (b), and pulsation rate vs lysosomal destabilization (c).

endpoint indicated that Cu caused significant cellular damage. Lysosomes play critical roles in nutrient processing and autophagic processes that are impaired in response to pollutant and temperature stress (Nardi et al., 2017; Ringwood, 2021). Increases in autophagy, another endpoint for lysosomal stress, were observed in microscopic analyses of *Pocillopora damicornis*, after exposure to high temperature (HT), ultraviolet (UV) and far-red (FR) radiation stress that increased coral tissue deterioration (Camaya et al., 2016). Significant linkages have been identified between lysosomal damage and reproductive success related to embryonic development in bivalves (Ringwood and Conners, 2000;

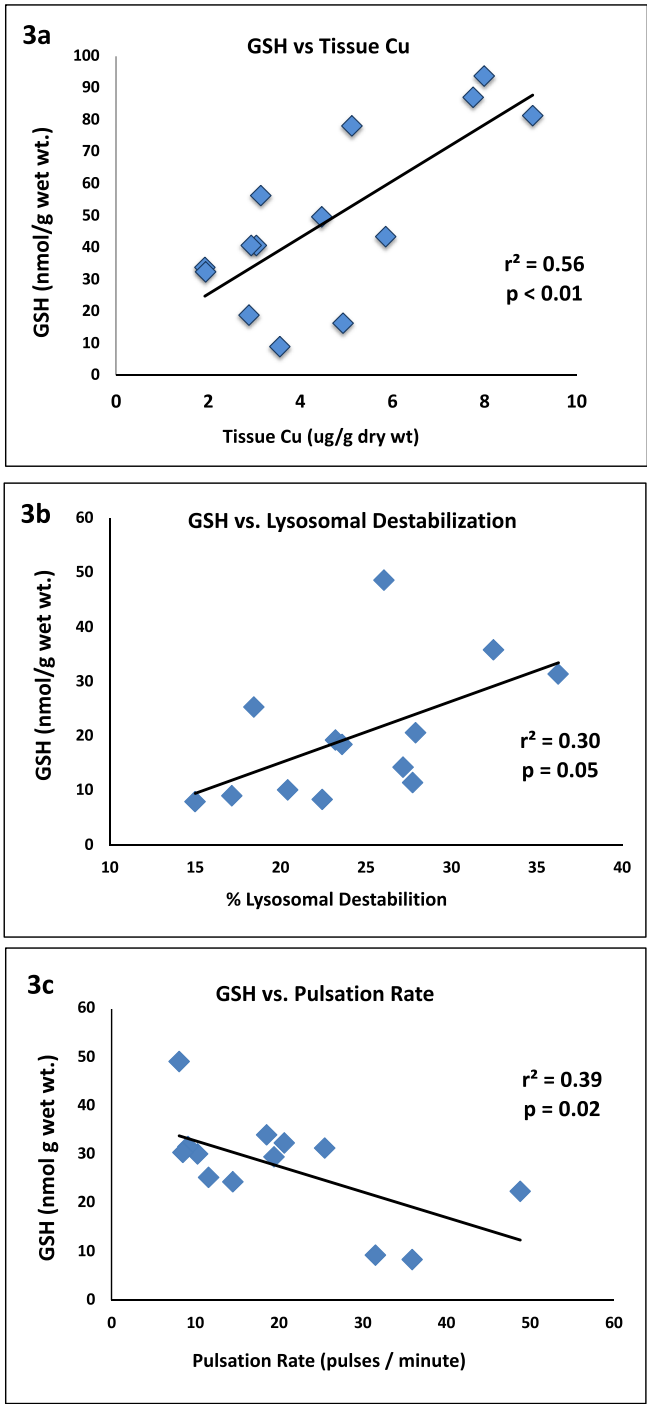

Fig. 3. *Aurelia aurita*, relationship between GSH concentrations and tissue Cu (a), GSH concentrations and lysosomal destabilization (b), and GSH concentrations and pulsation rate (c).

Moore et al., 2006), indicating the potential for population impacts.

These studies also demonstrates the potential value of behavioral endpoints, especially pulsation rates. Jellyfish lysosomal destabilization and pulsation rates were highly correlated to tissue Cu accumulation and to each other after 48 hour exposures. Pulsation rates have also served as valuable sensitive endpoints in other studies with larval stages of moon jellyfish and another valuable species, *Cassiopea* sp., known as the upside down jellyfish (Tills et al., 2016; Templeton et al., 2021). In studies with anemones, *Exaiptasia pallida*, tentacle retraction was used as a behavioral endpoint when exposed to Cu and Zn; the 24 hr EC50s for Cu were approximately 40 μg/L (Ianna et al., 2020). Similar to our results for jellyfish where the 24 hr EC50 for pulsation rate was approximately 25 μg/L, these studies indicate that behavioral endpoints can be very sensitive assays.

After only 24 hours, 100% mortality was observed in Jellyfish exposed to 50 μg/L Cu. Studies with the developmental stages of *A. aurita* also indicate significant toxicity to Cu, but some may be slightly less sensitive than adults. In a study with polyps exposed to a low and high concentration of Cu (20 and 200 μg/L), mortality was approximately 90% after 4 days when exposed to 200 μg/L Cu (Lucas and Horton, 2014). Ephyrae, the larval form, exhibited 100% mortality after 24 hour exposures to 1.9 mg/L Cu (the lowest concentration tested in those studies) (Mercado et al., 2023). When gamete bundles from *Acropora surculosa* were exposed to Cu for 12 hours, none survived at concentrations above 58 μg/L (Victor and Richmond, 2005).

Significant correlations were observed between GSH levels and tissue Cu as well as lysosomal destabilization and pulsation rate. GSH levels of control jellyfish were very low, ranging from approximately 8–25 nmol/g. Glutathione is a fundamentally important tripeptide that directly impacts oxidative balance, and is also an important cofactor for antioxidant enzymes and detoxification mechanisms (Meister and Anderson, 1983; Regoli and Guiliani, 2014). Glutathione, the most abundant antioxidant, along with other important antioxidants such as catalase, superoxide dismutase, glutathione reductase, etc. serve to mitigate oxidative damage (Kelly et al., 1998; Fonseca et al., 2021a). An increase in GSH is induced by a significant increase in oxyradical production. But it is important to remember that when GSH production or regeneration is overwhelmed by pollutants, temperature, and other stressors, GSH depletion can occur, especially after longer durations, or in response to multiple stressors. Therefore, significant perturbations of antioxidant status can manifest as an increase in GSH, as cells initiate production of GSH in response to oxyradicals, or as a depletion of GSH when oxyradical production overwhelms and impairs GSH production. A common scenario is an initial increase in GSH to cope with increased oxyradical production, but if sufficient production of GSH and other antioxidants cannot be sustained, depletion will be observed. The impacts of GSH depletion on other cellular and physiological processes are well documented, from the mammalian to invertebrate realms, and they include increased cellular damage, reduced reproductive success, and effects on immune responses (Meister and Anderson, 1983; Conners and Ringwood, 2000; Ringwood and Conners, 2000; Mello et al., 2020). Stress factors that affect immune responses in corals will likewise lead to increases in disease, further weakening the ability of corals to recover from a bleaching or exposure event (Palmer and Traylor-Knowles, 2012; Silva-Lima et al., 2021). We chose Cu for these studies because it is a classic example of a pollutant that causes oxidative damage related to Fenton chemistry and generation of hydroxyl radicals (Kelly et al., 1998; Halliwell and Gutteridge, 2015). In our short term Cu exposure studies reported in this paper with jellyfish, increases in GSH were observed, indicating induction of GSH in response to increased oxyradical production. In studies with a related jellyfish species, polyps of *A. coerulea* exposed to Cu exhibited increases in oxidative stress (based on a suite of biomarker assays) and alterations in glutamate pathways (based on genomic studies) (Liu et al., 2024). While GSH was not measured in their studies, increases in the glutamate pathways related to GSH synthesis were observed. The polyps are an important reproductive stage and in Liu et al. (2024) studies, budding and other developmental processes were impaired due to oxidative stress. The Cu levels used in those studies were much higher (0.1-10 mg/L), and while few polyps thrived above 0.1 mg/L, this stage may be a little less sensitive than the medusae stage. In a study with the Caribbean coral *Mussismilia harttii* exposed to Cu, thermal stress and a combination of these two stressors, GSH depletion and reductions in antioxidant enzymes were reported (Fonseca et al., 2017), an outcome that would be expected when antioxidant capacity is exceeded.

Therefore, in addition to lysosomal damage and changes in GSH status, significant impacts on jellyfish pulsation rates as well as

Table 2
Stress responses in corals exposed to Cu, pyrene, and elevated temperature for 48 hours. The Xs indicate bleaching (1 X indicates moderate bleaching and 2 XXs indicate severe bleaching). M is used to indicate excess mucus production (1 M indicates moderate mucus production and 2 Ms indicate heavy mucus production). ne indicates no effects

Coral Species	Control	Cu (ug/L) (50)	Cu (ug/L) (200)	Pyrene (ug/L) (10)	Pyrene (ug/L) (100)	Temp. Stress
Acropora divaricata	ne	ne	X	XX	XX	XX
Montipora capricornis	ne	X	XX	X	X	ne
Caulastrea furcata	ne	M	MM	ne	X	ne

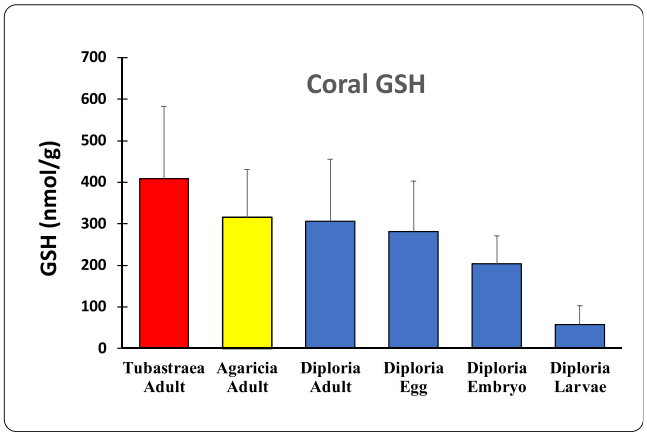

Fig. 4. GSH concentrations of three species of corals collected from Curacao

swimming and feeding behaviors were observed. The cellular responses to Cu exposures translated into impacts on physiology and organismal behavior, indicating impacts based on multiple levels of biological organization

When the three coral species used for these studies were exposed to Cu, no bleaching was observed in *Caulastrea furcata* or *Acropora divaricata* exposed to 50 µg/L Cu, but moderate bleaching was observed in *Montipora capricornis* after 48 hours. *A. divaricata* was however very sensitive to an acute increase in temperature. Branching corals of this genus have experienced serious bleaching events caused by rising sea surface temperatures (Weis, 2008; Woesik et al. 2022). *Acropora divaricata* was also much more sensitive to pyrene than Cu, as severe bleaching was observed at 10 µg/L pyrene. Species-specific differences in Cu uptake, sensitivities, and biochemical responses were found when three different species (*Acropora cervicornis, Pocillopora damicornis*, and *Montastraea faveolata*) were exposed to low Cu concentrations over a 5 week period (Bielmyer et al., 2010). Nevertheless, jellyfish adults were more sensitive than coral adults to Cu exposures in our studies. In studies with coral larvae of two species, *A. millepora* and *A. tenuis*, exposed to Cu, the threshold for successful metamorphosis was estimated as 25-30 µg/L, e.g. Cu levels above these concentrations resulted in a decrease from 80% to 50% success (Negri and Hoogenboom, 2011). These Cu effects concentrations on larvae are similar to those observed with the adult jellyfish pulsation and lysosomal destabilization assays (e.g. significant toxicity at 25 µg/L).

These studies also provide important baseline information on GSH levels in corals and jellyfish. The GSH levels of jellyfish (<25 nmol/g) were lower than those observed in adult corals, but tended to be more similar to those observed in larval corals. The GSH levels of adults of the three species of Carribbean corals, *Diploria labyrinthiformis, Tubastraea coccinea*, and *Agaricia humilis* were in the range of 300-500 nmol/g. The GSH levels of *D. labyrinthiformis* larvae were much lower than adults and eggs, approximately 57 nmol/g. The early developmental stages are non-feeding stages, and developmental changes can generate significant oxyradical production. Starvation is also associated with GSH depletion, so non-feeding life history stages experience decreases in GSH. Developmental stages that have low baseline GSH levels such as larval stages, may be more sensitive than adults and other stages to pollutant stress.

Adults of all of these Cnidarian species and the developmental stages have much lower GSH levels than those observed in more commonly used indicator species. The GSH levels of mussels and oysters tend to be in the range of 1000 - 1500 nmol/g, and fish levels are higher (Khan and Ringwood, 2016). Anemones are also regarded as a valuable surrogate species for characterizing potential risks to corals. The GSH levels of anemones in the range of 700 nmol/g have been reported (Mitchelmore et al., 2003), somewhat higher than corals, but lower than bivalves. Using GSH as an index of relative sensitivity, these studies suggest that more traditional bioindicator species that are typically used to assess potential toxicity of pollutants and stressors may not adequately reflect the potential risks to corals and other Cnidarians.

Another important aspect of coral sensitivity is the presence of symbiotic algae, zooxanthellae, in reef-building corals. One benefit of using *Aurelia aurita* is that they do not have zooxanthellae, so the results are more likely to reflect the animal tissue responses. We also found that the biomarkers (lysosomal destabilization and GSH levels) were readily conducted with pieces of bell tissue. Because this species of jellyfish does not have zooxanthellae and the bell tissues do not have nematocysts, the tissue preparations were easier. However, holobiont studies with corals and other Cnidarians that contain zooxanthellae, such as anemones and *Cassiopea*, that identify the impacts of stressors on both members of the symbiotic relationship are very important. Indeed, some studies have reported the responses of both, and their potential contribution to stress responses (Mitchelmore et al., 2007; Bielmyer et al., 2010; Brock and Bielmyer, 2013; Fonseca et al., 2021b; Cotinat et al., 2022).

The Cu toxicity studies reported here for jellyfish and corals as well as the GSH studies indicate that jellyfish, which may be as sensitive or even slightly more sensitive than corals, can serve as a valuable bioindicator species for coral toxicology assessments. *Aurelia aurita* is readily cultured in large numbers by public science institutions as well as academic and aquaculture facilities. They are also widely distributed and sometimes occur in very large numbers, so field collected jellyfish could also be used for toxicity studies. Our studies have also demonstrated lysosomal destabilization assays, which are regarded as valuable sensitive toxicity tools for a variety of bioindicator species worldwide, can be readily used with jellyfish. The sensitivity of the behavioral response of pulsation rate can provide a means of rapid assessment of a variety of chemicals such as sunscreens and personal care products as well as other metal and organic pollutants. Jellyfish could also be exposed to water samples collected from field sites to assess potential toxicity following an environmental event. These non-destructive approaches can also be used as part of citizen science programs and

educational engagement.

Cultivated as well as wild corals are precious for habitat restoration programs so having sufficient materials for toxicity testing is a challenge, and commonly used bioindicator species such as bivalves and fish may not adequately reflect potential toxicity. Toxicological studies with a broad range of invertebrate taxa are essential for the development of ecologically relevant assessments for natural populations (Rosner et al., 2024). The fundamental studies reported in this paper for jellyfish and corals contribute to the growing body of toxicological research on jellyfish, corals, and other Cnidarians, and provide guidance for the continued development of valuable screening tools for protecting coral reefs and sensitive marine habitats.

CRediT authorship contribution statement

A.H. Ringwood: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **M. Lowder:** Writing – original draft, Investigation, Conceptualization. **E. Provance:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **J. O'Dea:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **T. Gaspar:** Writing – original draft, Project administration, Methodology. **K.R.W. Latijnhouwers:** Resources, Methodology, Investigation, Conceptualization. **V.F. Chamberland:** Resources, Methodology, Investigation, Conceptualization. **M.J.A. Vermeij:** Methodology, Investigation, 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.2025.107265.

Data availability

Specific data may be requested.

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