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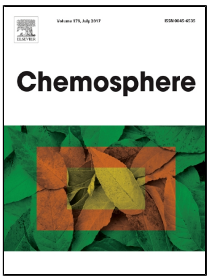

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Herbicide Effects on the Growth and Photosynthetic Efficiency of *Cassiopea maremetens*

David J. Rowen[1], Michelle A. Templeman[2*] & Michael J. Kingsford[1,3]

[1] College of Marine & Environmental Sciences, James Cook University, Townsville, QLD 4811, Australia; [2] TropWATER, James Cook University Townsville, Queensland, 4811, Australia; [3] ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia. *Corresponding author

Abstract

Herbicides from agricultural run-off have been measured in coastal systems of the Great Barrier Reef over many years. Non-target herbicide exposure, especially photosystem II herbicides has the potential to affect seagrasses and other marine species. The symbiotic benthic jellyfish *Cassiopea maremetens* is present in tropical/sub-tropical estuarine and marine environments. Jellyfish (n=8 per treatment) were exposed to four separate concentrations of agricultural formulations of diuron or hexazinone to determine their sensitivity and potential for recovery to pulsed herbicide exposure. Jellyfish growth, symbiont photosynthetic activity and zooxanthellae density were analysed for herbicide-induced changes for 7 days followed by a 7 day recovery period. Both the jellyfish and endosymbiont were more sensitive to diuron than hexazinone. The 7-day EC_{50} for jellyfish growth was 0.35 $\mu g.L^{-1}$ for Diuron and 17.5 $\mu g.L^{-1}$ for Hexazinone respectively. Diuron exposure caused a significant decrease ($p<0.05$) in jellyfish growth at 0.1 $\mu g.L^{-1}$, a level that is below the regional Great Barrier Reef guideline value. Jellyfish recovery was rapid with growth rates similar to control animals following removal from herbicide exposure. Both diuron and hexazinone caused significant decreases in photosynthetic efficiency (effective quantum yield) in all treatment concentrations (0.1 $\mu g.L^{-1}$ and above) and this effect continued in the post-exposure period. As this species is frequently found in near-shore environments, they may be particularly vulnerable to herbicide run-off.

Key Words: Jellyfish, Inhibition, Great Barrier Reef, Diuron, Hexazinone, Zooxanthellae

Highlights:

- Jellyfish are more sensitive to Diuron than Hexazinone;
- Diuron effects occurred at concentrations below current Great Barrier Reef guideline levels;
- Hexazinone caused post-exposure inhibition in zooxanthellae density;
- Jellyfish recovery was more rapid than symbiont recovery during the post-exposure period.

1. Introduction

Herbicides are an integral component of global agriculture. However, inappropriate and overuse of many herbicides can affect non-target species particularly in aquatic ecosystems. Herbicides and their degradation products have been detected in coastal ecosystems globally (e.g. Ali et al. 2014; Nodler et al. 2014; Caquet et al. 2013; Munaron et al. 2012). In tropical Australia, herbicides have been measured in coastal locations including the Great Barrier Reef (GBR) lagoon and are commonly associated with agricultural run-off (Brodie et al. 2012; Davis et al. 2012; Fabricius 2005, Haynes et al. 2000). In tropical regions, the greatest potential for offsite impacts from agricultural run-off are often associated with the "first flush" heavy rainfall and flood events associated with intense weather systems (Davis et al. 2012). It has been estimated that up to 30,000 kg per year of herbicides can be transported from agricultural areas to the GBR during these events (Kroon et al. 2012; Waterhouse et al. 2012). Flushing of herbicides and their residues to coastal marine systems has been identified to affect the health and structure of seagrass and coral communities and their associated benthic invertebrate inhabitants (Negri et al. 2015; Flores et al. 2013).

Diuron (DCMU or (3-(3,4-dichlorophenyl)-1,1-dimethylurea)) and hexazinone (3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-dione) are widely used herbicides in Australia, particularly in sugarcane growing areas and are of concern for the GBR (Brodie et al. 2012; Lewis et al. 2012). Diuron is a phenylurea herbicide, and hexazinone is an *s*-triazine herbicide. Both are classified as photosynthesis inhibitors, specifically targeting photosystem II (PSII) of the photosynthetic complex in the thylakoid membranes of chloroplasts (Sandman and Bolger 1986). Diuron residues have been measured in GBR marine sediments at concentrations up to 10$\mu g.kg^{-1}$ (Haynes et al. 2000a; Müller et al. 2000). During wet season flows, diuron has been found in concentrations up to 8.5$\mu g.L^{-1}$ in the lower Burdekin River catchment (Davis et al. 2012). Hexazinone has typically been detected at lower concentrations (0.3$\mu g.L^{-1}$) but its continued use in agriculture still presents a potential risk to marine biota, particularly as it is frequently used in conjunction with other herbicides to target pre-emergent weeds (Davis et al. 2008; Mitchell et al. 2005).

The euryhaline scyphozoan jellyfish *Cassiopea maremetens* was selected as the target organism as it can be found in lower estuarine systems where salinity is greater than 12 ppt. Recent studies (Epstein et al. 2016, Klein et al. 2016) have demonstrated that this species responds rapidly to pollutant exposure and is potentially a useful bioindicator of environmental stress. *C. maremetens* are found in warm coastal regions including sheltered lagoons, estuaries, mangroves, seagrass beds, coral reefs, and mudflats (Drew 1972). The symbiotic relationship with unicellular dinoflagellate zooxanthellae (*Symbiodinium* sp.) and sedentary nature make them useful as a target organism and potential proxy for coral species in tropical coastal waters.

The specific aims of this project were to: 1) assess herbicide effects on growth in *C. maremetens*; 3) assess herbicide effects on the endosymbiont zooxanthellae; and 4) assess the sensitivity of the jellyfish relative to measured values in in estuarine and coastal regions of the GBR.

2. Materials & Methods

C. maremetens polyps were sourced from Reef HQ in Townsville, Queensland with the parent stock originating from Lake Magellan, Sunshine Coast, Queensland, Australia. Strobilation was induced through halo-shocking, with ephyrae removed daily and placed in 10 litre plastic aquaria containing filtered (0.45µm) seawater and grown out for 3-4 weeks until they reached a minimum 10 mm diameter. Animals were maintained in filtered natural seawater at 33±1ppt salinity at 25±1^{0}C on a 12:12 light:dark cycle.

Herbicide stock solutions were prepared using agricultural grade Diurex^{TM} WG herbicide (900 g/kg Diuron) and Macspred Velmac^R G (200 g/kg Hexazinone). Agricultural grades were used as environmental risks posed by exposure to agricultural formulations can vary from analytical grade compounds due to the presence of trace levels of surfactants and other products used to improve wettability and mixing. Diurex is a water soluble granule while Velmac is typically applied as a granular herbicide to damp or wet ground with no pre-dilution.

Stock solutions were prepared by accurately weighing each formulation and dissolving in 1 L Milli-Q water to produce a 1.00 g.L^{-1} of the respective active ingredient stock solution. Treatment solutions were prepared

by diluting the appropriate volume of stock solution in the same filtered seawater used for culturing and rearing.

All equipment was cleaned by washing in phosphate-free detergent followed by multiple rinses in tapwater. Equipment was then soaked overnight in 10% AR-grade nitric acid followed by several rinses with Milli-Q water and allowed to air-dry before use.

A 96 hour pilot assessment of acute toxicity of the two herbicides was undertaken. Neither herbicide was acutely toxic to C. *maremetens* at concentrations up to 1000 $\mu g.L^{-1}$. However, EQY was inhibited at concentrations above 10 $\mu g.L^{-1}$ for both diuron and hexazinone and there was visible bleaching in the jellyfish tissues in the higher hexazinone concentrations (data not shown).

The 2014 study comprised 7 day herbicide exposure followed by 7 day recovery (filtered seawater only). Treatment concentrations bracketed reported "first flush" event concentrations and ecotoxicological studies on other species (e.g. Mitchell et al. 2005; Jones 2005; Davis et. al. 2008; 2012; Negri et al. 2015).

Each herbicide treatment and control comprised eight replicate animals randomly allocated to individual chambers containing 150 mL of the respective treatment or control solutions. Treatment concentrations comprised 0$\mu g.L^{-1}$ (Control), 0.1$\mu g.L^{-1}$, 0.5$\mu g.L^{-1}$, 5.0 $\mu g.L^{-1}$ and 30 $\mu g.L^{-1}$ diuron or 0 (Control), 0.1$\mu g.L^{-1}$, 2$\mu g.L^{-1}$, 15 $\mu g.L^{-1}$ and 40 $\mu g.L^{-1}$ hexazinone.

Photosynthetic yield was measured daily using a Heinz Walz GmbH Photosynthesis Yield Analyzer Mini-PAM Portable Chlorophyll Fluorometer (PAM). The PAM measures effective quantum yield ($\Delta F/F_m'$) of photochemical energy conversion though PSII from two consecutive measurements of fluorescence yield. Effective quantum yield (EQY) is directly proportional (1:1) to photosynthetic efficiency; therefore it is a simple metric to quantify the degree of photosynthetic activity (Magnusson et al. 2008). PAM measurements were performed in triplicate with animals light-adapted for at least 2 hours to ensure PSII sites were activated.

The bell diameter was measured at Day 0, 7 and 14 to the nearest millimetre during the extension phase of each pulse using a plastic ruler. Animals were fed daily and allowed to freely feed for approximately four hours before solutions were replaced.

On Day 7, four replicate animals from each treatment and the Control were removed, rinsed in clean, filtered seawater and placed in clean and labelled 30mL tubes, wrapped in alfoil and frozen at -18ºC for later zooxanthellae extraction. The remaining animals were fed as normal and after four hours feeding were removed from the old containers and placed in new, clean containers with 150 ml clean filtered seawater for the recovery phase. On Day 14 animals were rinsed in clean, filtered seawater, placed in clean, labelled 30mL tubes, wrapped in alfoil and frozen at -18ºC.

2.1 Zooxanthellae Density Counts

Intact zooxanthellae were extracted from frozen *C. maremetens* using a modified methodology from Zamoum & Furla (2012). The modified methodology used 0.5mL 1M NaOH solution per jellyfish sample with the final volume (after incubation) being standardised to 0.5mL. Zooxanthellae abundance was determined using an improved Neubauer haemocytometer. As the zooxanthellae in *C. maremetens* are typically contained within ambeocytes within the surface layers of the bell and oral arm tissues (Estes et. al. 2003), the final cell densities were standardised to the calculated surface area of bell tissue (mm^2).

2.2 Data Analyses

End point analyses at Day 7 (exposure) and Day 14 (recovery) were performed using univariate 1-way ANOVA using GraphPad Prism version 7.01 (California USA) for EQY (zooxanthellae), growth (jellyfish) and zooxanthellae abundance. ANOVA assumptions were tested using Bartlett's test and $\log_{10}$ transformed to meet assumptions if required. If data continued to violate assumptions despite transformation, a more conservative significance level (α=0.01) was adopted to reduce the possibility of performing a Type 1 error (Underwood 1997). All other data were tested using the standard significance level of α=0.05. Posteriori

Dunnett's multiple comparisons tests were also performed to determine differences between specific treatment groups at $p < 0.05$.

For determination of EC_{50} for each of the parameters (EQY, growth and zooxanthellae abundance) a four parameter nonlinear regression was fitted to normalized dose-response data using GraphPad Prism version 7.01 (California USA).

3. Results

Jellyfish growth was affected by exposure to both herbicides (Figure 1). Diuron affected growth of *C. maremetens* at all concentrations with changes in size significantly different from the Control ($F_{4,35}$=7.332, p<0.05) (Figure 1). A similar effect was seen in the hexazinone exposed animals although the degree of effect was different to the diuron exposed animals ($F_{4,35}$=5.448, p<0.05). Following removal from the exposure to the herbicides, jellyfish growth recovered to levels similar to the Control animals within seven days (Figure 1).

INSERT FIGURE 1 HERE

Figure 1. The change in size in *Cassiopea maremetens* at end of exposure to diuron or hexazinone and after seven days recovery in clean seawater. Data are mean ± 1 SE, n = 8 for exposure and n=4 for recovery. Significance level $p < 0.05$ represents difference from respective control and is indicated by *.

Exposure to both herbicides significantly decreased photosynthetic activity (EQY) in symbionts (Figure 2). Seven days exposure to diuron significantly reduced EQY in all concentrations ($F_{4,35}$=537, p<0.05) and caused almost complete photoinhibition at 5 and 30µg.L^{-1} respectively. Hexazinone exposure also significantly decreased EQY in all exposure concentrations ($F_{4,35}$=114, p<0.05) (Figure 2).

Photosynthetic activity increased during the recovery phase for all treatment jellyfish. However, there was still evidence of photosynthetic impairment after seven days post-exposure with all herbicide concentrations remaining significantly lower than Control activity for both diuron ($F_{4,15}$=144, p<0.05) and hexazinone

($F_{4,15}$=229, p<0.05) (Figure 2). Variation among replicates was also very low (<5% mean) across all treatments.

INSERT FIGURE 2 HERE

Figure 2. One-way ANOVA of EQY of the endosymbiont in *C. maremetens* at end of exposure to diuron or hexazinone and after seven days recovery in clean seawater. Data are mean ± 1 SE, n=8 for exposure and n=4 for recovery. Significance level $p < 0.05$ represents difference from respective control and is indicated by *

Herbicide exposure caused the expulsion of symbionts from the jellyfish tissue resulting in lower zooxanthellae densities (Figure 3). Zooxanthellae density in *C. maremetens* tissues decreased with increased herbicide exposure with differences significant at 30µg.L^{-1} diuron ($F_{4,15}$=4.988, p<0.05). Hexazinone exposure also induced a reduction in zooxanthellae density after 7 days in all concentrations however these were not significantly different from Control ($F_{4,15}$=3.784, p>0.01).

Following removal of the jellyfish from the herbicide exposure there was limited recovery in zooxanthellae density 7 days post-exposure particularly at the higher concentrations (Figure 3). Symbiont density in the diuron exposed animals were similar to, or lower than, that recorded at the end of the exposure period (Figure 3) with the density in the 30µg.L^{-1} group significantly different from Control animals ($F_{4,15}$=21.61, p<0.05). Similarly the hexazinone exposed animals demonstrated little in the way of zooxanthellae recovery with overall densities lower than densities at the end of the exposure period (Figure 3). At seven days post-exposure cell densities above 0.1 µg.L^{-1} hexazinone were significantly different from the Control animals ($F_{4,15}$=8.077, p<0.01).

INSERT FIGURE 3 HERE

Figure 3. The mean zooxanthellae density (mm^2) in *C. maremetens* at end of exposure to diuron or hexazinone and after seven days recovery in clean seawater. Data are mean ± 1 SE, n=4. Significance level $p < 0.05$ indicated by * $p < 0.01$ indicated by ** and represents difference from respective controls.

3.1 Dose-Response

A four parameter logistic equation with the maximum constrained to 100% was used to derive dose-response curves for diuron and hexazinone exposure for growth, EQY and zooxanthellae density using GraphPad Prism version 7.01 (Table 2). The EC_{50} values for diuron were lower for all responses than those calculated for hexazinone exposure, suggesting that *C. maremetens* and its symbionts are more sensitive to diuron than hexazinone. In diuron exposed animals, growth was the most sensitive endpoint followed by photosynthetic activity then zooxanthellae density. In contrast, for the hexazinone exposed jellyfish, zooxanthellae density was the most sensitive endpoint, with photosynthetic yield the least sensitive response (Table 2). Derived 7-day diuron EC_{50} for jellyfish growth, photosynthetic yield and zooxanthellae density were also compared to water quality guideline values for the Great Barrier Reef (GBRMPA 2010). At present there is only a low reliability trigger value of $1.2 \mu g.L^{-1}$ available for hexazinone for the GBR.

Table 2. 7-day EC_{50} (95% CI) for diuron and hexazinone on the growth, EQY, and zooxanthellae density in *C. maremetens* and comparison to water quality guidelines for the Great Barrier Reef (GBR) TV=Trigger value, - no TV currently derived (GBRMPA 2010)

	Diuron (µg.L^{-1})	**Hexazinone (µg.L^{-1})**
Bell Area (mm^2)	0.349 (0.05-2.7)	17.51 (4.4-225)
Effective Quantum Yield	1.42 (1.2-1.7)	40.92 (29-58)
Zooxanthellae Density (mm^2)	1.86 (0.36-9.1)	4.72 (0.43-29)
GBR 99% TV	0.9	-
GBR 95% TV	1.6	-
GBR 90% TV	2.3	-

4. Discussion

While neither diuron nor hexazinone were acutely toxic to *C. maremetens* during a 96 hour exposure, there were significant physiological effects on jellyfish growth and symbiont activity and survival. Diuron exposure caused physiological inhibition in both *Cassiopea maremetens* and its symbiont at concentrations that have been reported from estuarine and coastal regions of the GBR (Haynes et al. 2000a; Müller et al. 2000; Mitchell et al. 2005; Davis et al. 2008; Davis et al. 2012). Herbicide sensitivity differed between the two tested herbicides. The most sensitive endpoint to diuron was the jellyfish growth while the symbionts (zooxanthellae density) were more sensitive to hexazinone exposure (Table 2).

PSII herbicide effects on non-target species can exhibit a bi-modal toxic effect between autotrophic and heterotrophic species, with autotrophs exhibiting a higher sensitivity to herbicide exposure than heterotrophs (e.g. Jones 2005; Pathiratne and Kroon 2016). Traditionally, jellyfish are believed to be tolerant of environmental perturbations, but recent studies have shown that jellyfish, *Cassiopea* sp. in particular, is sensitive to pollutant exposure (Templeman and Kingsford 2015; Klein et al. 2016).The

sensitivity of *C. maremetens* to the herbicides was greater than many other heterotrophs, although similar effects have been found in other symbiotic cnidarians, particularly corals (e.g. Raberg et al. 2003; Cantin et al. 2007).

Herbicides from agricultural run-off have been measured in coastal systems of the Great Barrier Reef over many years. Non-target herbicide exposure, especially photosystem II herbicides has the potential to affect seagrasses and other marine species. The symbiotic benthic jellyfish *Cassiopea maremetens* is present in tropical/sub-tropical estuarine and marine environments. Jellyfish were exposed to agricultural formulations of diuron or hexazinone to determine their sensitivity and potential for recovery to pulsed herbicide exposure. Jellyfish growth, symbiont photosynthetic activity and zooxanthellae density were analysed for herbicide-induced changes for 7 days followed by a 7 day recovery period. Both the jellyfish and endosymbiont were more sensitive to diuron than hexazinone. The 7-day EC_{50} for jellyfish growth was 0.35 $\mu g.L^{-1}$ for Diuron and 17.5 $\mu g.L^{-1}$ for Hexazinone respectively. Diuron exposure caused a significant decrease ($p<0.05$) in jellyfish growth at 0.1 $\mu g.L^{-1}$, a level that is below the regional Great Barrier Reef guideline value. Jellyfish recovery was rapid with growth rates similar to control animals following removal from herbicide exposure. Both diuron and hexazinone caused significant decreases in photosynthetic efficiency (effective quantum yield) in all treatment concentrations (0.1 $\mu g.L^{-1}$ and above) and this effect continued in the post-exposure period. As this species is frequently found in near-shore environments, they may be particularly vulnerable to herbicide run-off.

4.1 Jellyfish Post-Exposure Response

Recovery of *C. maremetens* growth post-exposure indicated a level of resilience once the chemical assault was removed. In algae, both *Navicula* sp. and *N. pyriformis* exhibited recovery in biomass at 5 and 10 days exposure to PSII herbicides including diuron and hexazinone (Magnusson et al. 2008). This was hypothesised to be a rescue effect through increased heterotrophic energy acquisition as has been seen in some algal species under limiting light conditions (e.g. Tuchman et al. 2006). As *C. maremetens* received complementary heterotrophic feeding during the study, a shift to a higher level of heterotrophically derived

nutrient could occur when endosymbionts are inhibited. Other cnidarians including sea anemones and scleractinian corals, have also shown the capacity to modify energy budgets by incorporating a larger proportion of heterotrophic sourced energy when under stress (e.g. Grottoli et al. 2006; Hoogenboom et al. 2010; Fransolet et al. 2014). Additionally, in many symbiotic cnidarians, immediate metabolic requirements are usually met by heterotrophic nutrition, with the energy derived from photosynthetic symbionts stored for energy intensive events including reproduction (Anthony and Fabricius 2000; Cantin et al. 2007). A similar energy acquisition scheme may be present in *C. maremetens* and may shift to incorporate more heterotrophy during symbiont stress or inhibition. However, under field conditions, the ability to compensate using heterotrophic inputs will be heavily dependent on local quantities and quality of food.

4.2 Symbiont Response

Photosynthetic inhibition was observed in all herbicide exposed *C. maremetens* with almost complete inhibition in 30 $\mu g.L^{-1}$ diuron (Figure 2). These results are consistent with studies on microalgae and corals (Cantin et al. 2007; Magnusson et al. 2008). Photosynthetic yield exhibited some recovery post-exposure to the herbicides. This indicated that the photosynthetic ability of zooxanthellae exhibited some level of resilience to PSII exposure. However, recovery was incomplete and significant inhibition was still present at all concentrations 7 days post-exposure (Figure 2).

Studies on corals have shown similar responses (Jones and Kerswell 2003; Negri et al. 2005; Cantin et al. 2007). Diuron binds reversibly to the photosynthetic complex and the impacted organism could be expected to recover once transferred to clean seawater (Jones and Kerswell 2003; Owen et al. 2003). Recovery rates will be dependent on a combination of repair of the PSII apparatus and/or the expulsion of damaged zooxanthellae but would depend on exposure duration for the capacity to recover. The exact mechanism of herbicide accumulation and detoxification in the *Cassiopea maremetens* symbiosis is still yet to be explored but recovery appears to be slower compared to other cnidarians (e.g. Jones and Kerswell 2003; Negri et al. 2005; Cantin et al. 2007).

279 In-tissue zooxanthellae densities decreased with increasing herbicide concentration exposure. In contrast to
280 the recovery in photosynthetic yield, however, there was minimal post-exposure recovery in zooxanthellae
281 density (Figure 3), with zooxanthellae densities continuing to decrease post-exposure in the higher
282 hexazinone concentrations.

283 The contrasting results between zooxanthellae density and yield appear to demonstrate an inconsistency
284 between the recovery responses of the symbiont. While there was improvement in effective quantum yield
285 post exposure, zooxanthellae density did not increase. This discrepancy between yield recovery and
286 zooxanthellae density is a product of the effective quantum yield measurement technique. The PAM
287 fluorometer detects the ratio between minimal and maximal fluorescence and accordingly it is not a density-
288 dependent response. As such, although zooxanthellae density did not increase and for the higher hexazinone
289 concentrations continued to decrease, the PAM data reflected the relative measure of the photosynthetic
290 capacity of the zooxanthellae still present within the tissues. This may help explain why photosynthesis
291 recovered to a greater extent while zooxanthellae density remained depressed through the recovery phase.

292 **4.3 Comparison to Regional Water Quality Guidelines**

293 Jellyfish growth (EC_{50}) was affected at diuron concentrations below the Great Barrier Reef Marine Park
294 Authority Water Quality Guidelines (GBRMPA WQG) 99% trigger value for protection of marine species
295 (0.9 $\mu g.L^{-1}$), while EC_{50} for photosynthetic yield and zooxanthellae abundance were observed at
296 concentrations below the 95% (1.6 $\mu g.L^{-1}$) and 90% (2.3 $\mu g.L^{-1}$) trigger values respectively (GBRMPA
297 2010). Although diuron was not acutely toxic to *C. maremetens*, there was significant growth inhibition at
298 all concentrations (Table 2, Figure 1). Hexazinone exposure also inhibited both host and symbiont,
299 however, the calculated EC_{50} was higher than measured concentrations in the lower riverine and coastal
300 zones of the GBR and above the GBRMPA WQG low reliability trigger value of 1.2 $\mu g.L^{-1}$ (GBRMPA
301 2010). These results demonstrate that *C. maremetens* is very sensitive to herbicide exposure.

302

5. Conclusions

The exposure duration of this study was designed to reflect exposure to short-term pulse exposure from flood plume type events that are consistent with wet season flood events into the GBR lagoon. However, wet season flows to the GBR are typically considered to be longer 'press' events which can result in much longer exposure periods to pollutants. While this study demonstrated recovery in EQY upon removal from the herbicide exposure, the zooxanthellae density continued to demonstrate inhibition during the post-exposure period. Longer exposure periods and / or repeated 'pulse' exposures may result in a reduced capacity for recovery particularly if exposure is combined with other stressors including reduced salinity and increased turbidity that are commonly associated with flood plume events.

C. maremetens was shown to be sensitive to the herbicides diuron and hexazinone although the response to herbicide exposure varied between the two compounds and the response measure. The diuron concentrations eliciting a response were comparable with ambient concentrations measured in coastal zones of the GBR and lower than the current GBRMPA trigger value. Hexazinone was less toxic to the jellyfish however, recovery in zooxanthellae density was limited indicating there was ongoing reduced physiological fitness in the host jellyfish. Overall, *C. maremetens* is sensitive to herbicide exposure at environmental concentrations measured in coastal regions of the GBR, and may be particularly vulnerable to pulse exposures from flood plumes.

Conflict of Interest / Ethics

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

The authors declare they have no conflict of interest.

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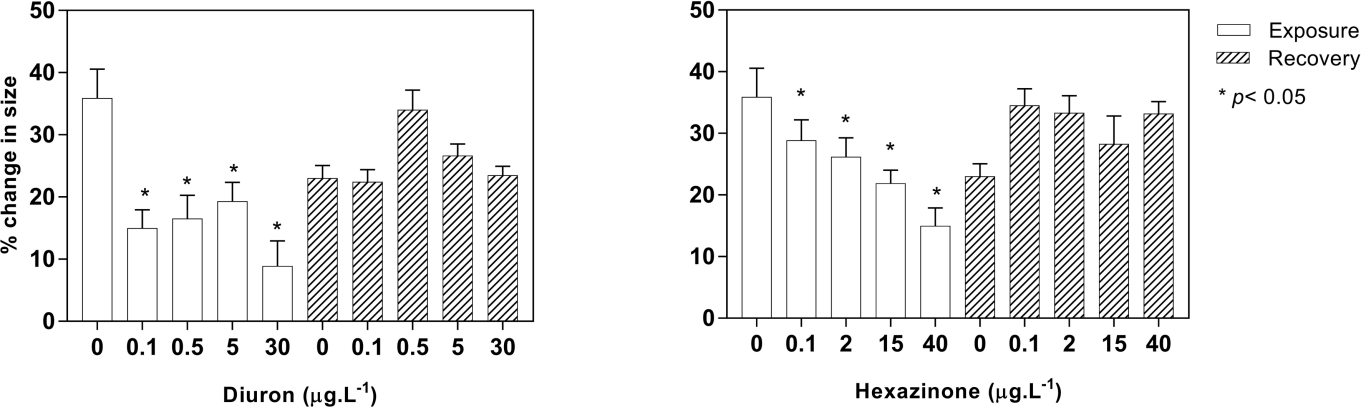
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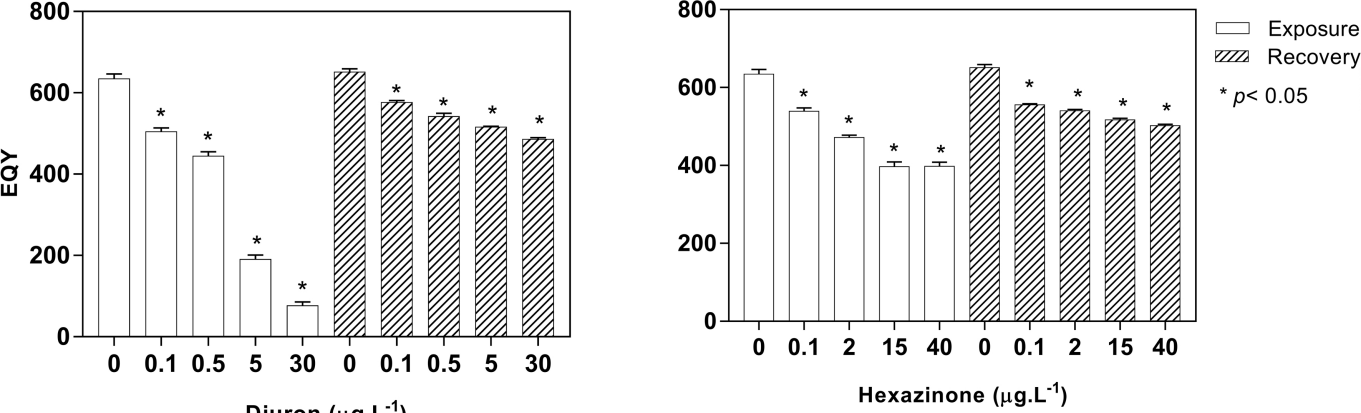
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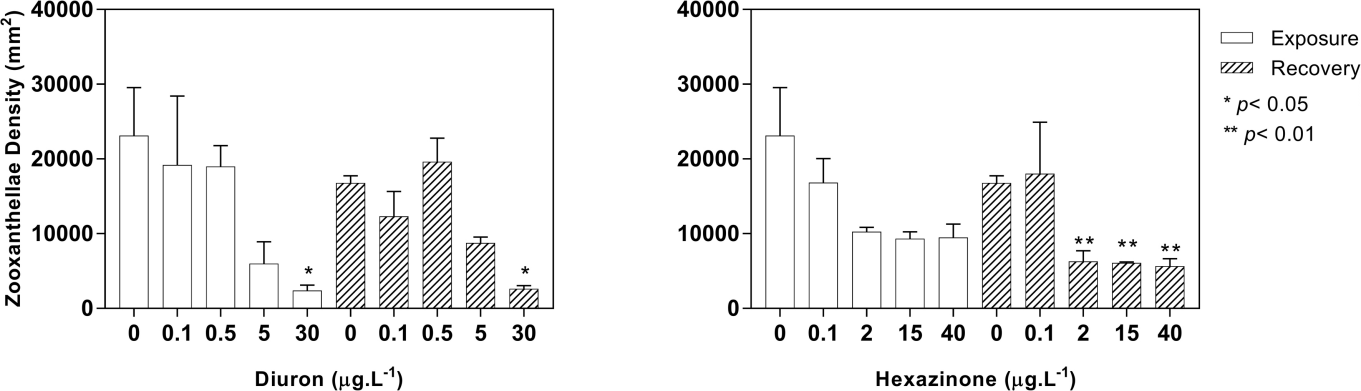
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Exposure
Recovery
* p< 0.05
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