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Herbicide effects on the growth and photosynthetic efficiency of *Cassiopea maremetens*

David J. Rowen [a], Michelle A. Templeman [b, *], Michael J. Kingsford [a, c]

[a] College of Marine & Environmental Sciences, James Cook University, Townsville, QLD 4811, Australia
[b] TropWATER, James Cook University Townsville, Queensland, 4811, Australia
[c] ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia

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.

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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 μg L^{-1} for Diuron and 17.5 μg L^{-1} for Hexazinone respectively. Diuron exposure caused a significant decrease ($p < 0.05$) in jellyfish growth at 0.1 μg 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 μg 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.

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

* Corresponding author.

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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 μg kg^{-1} (Haynes et al., 2000; Müller et al., 2000). During wet season flows, diuron has been found in concentrations up to 8.5 μg L^{-1} in the lower Burdekin River catchment (Davis et al., 2012). Hexazinone has typically been detected at lower concentrations (0.3 μg 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 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 L plastic aquaria containing filtered (0.45 μm) 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±1 ppt salinity at 25 ± 1 °C on a 12:12 light:dark cycle.

Herbicide stock solutions were prepared using agricultural grade Diurex™ 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 h pilot assessment of acute toxicity of the two herbicides was undertaken. Neither herbicide was acutely toxic to *C. maremetens* at concentrations up to 1000 μg L^{-1}. However, EQY was inhibited at concentrations above 10 μg 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 μg L^{-1} (Control), 0.1 μg L^{-1}, 0.5 μg L^{-1}, 5.0 μg L^{-1} and 30 μg L^{-1} diuron or 0 (Control), 0.1 μg L^{-1}, 2 μg L^{-1}, 15 μg L^{-1} and 40 μg 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 h 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 4 h 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 30 mL tubes, wrapped in alfoil and frozen at −18 °C for later zooxanthellae extraction. The remaining animals were fed as normal and after 4 h 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 30 mL tubes, wrapped in alfoil and frozen at −18 °C.

2.1. Zooxanthellae density counts

Intact zooxanthellae were extracted from frozen *C. maremetens* using a modified methodology from Zamoum and Furla (2012). The modified methodology used 0.5 mL 1 M NaOH solution per jellyfish sample with the final volume (after incubation) being standardised to 0.5 mL. 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