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Lethal and sub-lethal chronic effects of the herbicide diuron on seagrass

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ABSTRACT

Photosystem II herbicides from agricultural sources have been detected throughout nearshore tropical habitats including seagrass meadows. While PSII herbicides have been shown to inhibit growth in microalgae at low concentrations, the potential impacts of chronic low concentration exposures to seagrass health and growth have not been investigated. Here we exposed two tropical seagrass species *Halodule uninervis* and *Zostera muelleri* to elevated diuron concentrations (from 0.3 to 7.2 $\mu g\,l^{-1}$) over a 79-day period followed by a 2-week recovery period in uncontaminated seawater. PAM fluorometry demonstrated rapid effect of diuron on photosystem II (PSII) in both seagrass species at 0.3 $\mu g\,l^{-1}$. This effect included significant inhibition of photosynthetic efficiency ($\Delta F/F_m'$) and inactivation of PSII (F_v/F_m) over the 11 week exposure period. Significant mortality and reductions in growth was only observed at the highest exposure concentration of 7.2 $\mu g\,l^{-1}$ diuron. However, biochemical indicators demonstrated that the health of seagrass after this prolonged exposure was significantly compromised at lower concentrations. For example, the drop in C:N ratios (0.6 $\mu g\,l^{-1}$) and reduced $\delta^{13}C$ (1.7 $\mu g\,l^{-1}$) in seagrass leaves indicated reduced C-assimilation from photosynthesis. Critically, the energetic reserves of the plants (as measured by starch content in the root-rhizome complex) were approximately halved following diuron exposure at and above 1.7 $\mu g\,l^{-1}$. During the 2-week recovery period, the photosynthetic capacity of the seagrass improved with only plants from the highest diuron treatment still exhibiting chronic damage to PSII. This study shows that, although seagrass may survive prolonged herbicide exposures, concentrations $\geq$0.6 $\mu g\,l^{-1}$ diuron equivalents cause measureable impacts on energetic status that may leave the plants vulnerable to other simultaneous stressors. For example, tropical seagrasses have been heavily impacted by reduced light from coastal flood plumes and the effects on plant energetics from light limitation and diuron exposure (highest in flood plumes) are very similar, potentially leading to cumulative negative effects.

1. Introduction

1.1. Developmental pressures on tropical coastal ecosystems

By 2050 more than half of the global population is expected to live in the tropics (State of the Tropics, 2014), increasing the risk of coastal contamination with agrochemicals (Lacher and Goldstein, 1997). The Great Barrier Reef (GBR) is one of the best studied tropical coastal zones extending over 2000 km along the coast of northeast Australia. Its ecological significance at a global scale is acknowledged through its World Heritage listing; however the ecosystem services provided by the GBR are inextricably linked to those of its adjacent catchments (Stoeckl et al., 2011). Pollutants entering the GBR lagoon are predominantly catchment-derived and arise from economically important land-uses, such as grazing and agriculture crops (Bartley et al., 2014; Waterhouse et al., 2012).

1.2. Herbicides and the Great Barrier Reef

Herbicide usage within GBR catchments has increased by up to 7-fold in recent decades (Lewis et al., 2009) and it is estimated that over 30,000 kg of herbicides are introduced each year to the GBR via agricultural run-off (Kroon et al., 2012). Photosystem II

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(PSII) herbicides including diuron and atrazine are the most frequently detected in the GBR lagoon (Lewis et al., 2009; Shaw et al., 2010; Smith et al., 2012). This class of herbicide acts by binding to the D1 protein of photosystem II inhibiting electron flow, which in turn, limits carbon fixation in plants (Oettmeier, 1992). PSII herbicides are applied in crops and horticulture to target grass weeds often prior to periods of irrigation or rainfall which can in turn lead to concentrations of up to 8.5 $\mu g\,l^{-1}$ diuron in creeks that flush into the GBR (Davis et al., 2013). Concentrations of PSII herbicides within the coastal zone of the GBR lagoon can exceed 1 $\mu g\,l^{-1}$ (Lewis et al., 2009; Lewis et al., 2012), higher than GBR guideline values (GBRMPA, 2010). Since D1 is a highly conserved protein (i.e., it has been retained despite speciation), PSII herbicides also affect non-target organisms including marine phototrophs, such as seagrasses (Flores et al., 2013; Haynes et al., 2000; Macinnis-Ng and Ralph, 2004).

1.3. Seagrasses and herbicides

Highly productive and ecologically important seagrass ecosystems have become threatened globally due to increasing anthropogenic pressures (Orth et al., 2006; Waycott et al., 2009). One of the key pressures on seagrasses and other benthic species in tropical systems is severe light limitation for primary productivity caused by the large amounts of suspended solids delivered during the heavy rainfall periods in summer months (Collier et al., 2012a; Fabricius et al., 2014). PSII herbicides can also limit primary production in seagrass by reducing photosynthetic efficiency and/or causing damage to PSII (Flores et al., 2013; Haynes et al., 2000; Ralph, 2000; Seery et al., 2006). Since herbicide exposure peaks during flood periods (Lewis et al., 2009; Smith et al., 2012) there is mounting concern that persistent low concentrations of PSII herbicides in nearshore waters of the GBR may contribute to seagrass decline (Waterhouse et al., 2012; Waterhouse et al., 2013).

The effects of diuron on the photosynthetic capacity of seagrass can be measured at concentrations of 0.1–0.5 $\mu g\,l^{-1}$ (Chesworth et al., 2004; Flores et al., 2013; Haynes et al., 2000; Macinnis-Ng and Ralph, 2004; Ralph, 2000) which can be found in the GBR lagoon (Lewis et al., 2009; Lewis et al., 2012; Smith et al., 2012). The most sensitive indicator for PSII herbicide effects acts are measurements of photosynthetic condition made using pulse amplitude modulation (PAM) fluorometry. In particular the inhibition of effective quantum yield of PSII ($\Delta F/F_m'$) and maximum quantum yield (F_v/F_m) by herbicides that target PSII are highly responsive and biologically meaningful as they indicate reduction in the photosynthetic capacity and efficiency of the plant which can have flow-on effects to plant energetics and health (Gao et al., 2011,b; Ralph et al., 2007a,b). In light-limiting scenarios the effect of PSII herbicides is to severely limit carbon fixation (Johnson and Bird, 1995), while in high irradiance scenarios the blocking of electron transport pathways by PSII herbicides increases oxygen radical formation, causing additional chronic photoinhibition and damage to PSII (Genty et al., 1989; Osmond et al., 1999; Ralph and Burchett, 1995). Limited data also suggests that growth in seagrass seedlings (Gao et al., 2011) and mature plants (Johnson and Bird, 1995; Mitchell, 1987) can be significantly reduced by chronic exposures of atrazine. Plant-scale impacts, such as reductions in growth and abundance, have flow-on effects that could diminish their ecological function as productive sources of food, habitat and as nutrient sinks.

The acute sensitivity of photophysiological processes in seagrass to PSII herbicides is similar to that of tropical microalgae and corals (Flores et al., 2013). While exposure of microalgae to PSII herbicides leads to proportional reductions in $\Delta F/F_m'$ and growth (Magnusson et al., 2008), similar relationships for chronic seagrass exposure require further elucidation. Since the primary impacts of light and PSII herbicide exposure both centre on the function of PSII and the flow-on effects from reduced photosynthetic C incorporation, we assessed the chronic impacts of diuron exposure on seagrass under moderate light conditions expected in flood plumes. A set of biomarkers was assessed to link productivity declines in a long-term experimental exposure of seagrasses to diuron (Table 1). Clarifying the associations between these impacts represents an important step toward understanding the potential chronic effects of PSII herbicides on the health of seagrass meadows. It also provides insight into the likely cumulative effects of the co-occurring stressors, herbicides and low light.

2. Methods

2.1. Experimental approach

In the present study we exposed two tropical seagrass species to four elevated concentrations of diuron. The plants were potted in natural sediments with the exposure conducted under continuous flow-through conditions for 11 weeks, followed by a two week recovery period in uncontaminated water (the sediments were not renewed). The photosynthetic performances of plants were assessed throughout the exposure using PAM fluorometry while other biomarkers and growth were assessed at the end of the exposure period. The biomarkers used to assess the chronic effects of diuron are outlined in Table 1. The stress response caused by low light is described, as well as known or predicted responses to diuron for these biomarkers. Herbicide exposure was expected to affect PSII and pigments in seagrasses in the opposite manner to low light stress. However, as both low light and diuron result in lower energetic surplus, the flow-on effects at the plant-scale could be similar.

2.2. Plant collection

Halodule uninervis Ascherson (Cymodoceaceae) is a tropical seagrass species distributed throughout the Indo-West Pacific and *Zostera muelleri* Irmisch ex Ascherson (Zosteraceae), (syn *Zostera capricorni*) is a tropical to temperate species found in Australia and New Zealand (Waycott et al., 2004). Both occur in north-eastern Australia and the Great Barrier Reef (GBR). *H. uninervis* and *Z. muelleri* were collected from intertidal seagrass beds (<1 m) from Cockle Bay Magnetic Island (19° 10.88′S, 146° 50.63′E) and Pelican Banks, Gladstone, Australia (23° 46.005′S, 151° 18.052′E), respectively. *H. uninervis* and *Z. muelleri* plants were collected using a hand trowel, sealed in plastic bags with seawater and placed into 10 cm-diameter plastic pots for transport to the Australian Institute of Marine Science (AIMS) in Townsville, Australia.

Sixty pots of each of *H. uninervis* and *Z. muelleri* were maintained in outdoor aquaria (1000 l) with flow-through filtered seawater (5 μm) under 70% shading (maximum 350 μmol photons $m^{-2}\,s^{-1}$), ambient temperature (23–25 °C) and salinity at 35–36 PSU. Plants were transferred into indoor flow-through experimental tanks and allowed to acclimate for at least one week before experimentation. Prior to experimentation, *Z. muelleri* and *H. uninervis* plants (with approximately 20 shoots each) were re-planted with a mixture of their original sediment and silica beach sand. *Z. muelleri* were planted into 700 ml plastic pots (10 cm diameter; 9.5 cm deep) modified with 2 cm holes and fitted with pool skimmer socks while *H. uninervis* plants were re-planted into 1000 ml plastic experimental units (16 × 12.5 cm, 5.5 cm depth) and fitted with skimmer socks. The pot sizes reflected surface space needed for growth of each species, while the holes and skimmer socks allowed some water exchange through the sediment.