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Reduced salinity increases susceptibility of zooxanthellate jellyfish to herbicide toxicity during a simulated rainfall event

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ABSTRACT

Accurately predicting how marine biota are likely to respond to changing ocean conditions requires accurate simulation of interacting stressors, exposure regimes and recovery periods. Jellyfish populations have increased in some parts of the world and, despite few direct empirical tests, are hypothesised to be increasing because they are robust to a range of environmental stressors. Here, we investigated the effects of contaminated runoff on a zooxanthellate jellyfish by exposing juvenile *Cassiopea* sp. medusae to a photosystem II (PSII) herbicide, atrazine and reduced salinity conditions that occur following rainfall. Four levels of atrazine (0ngL^{-1}, 10ngL^{-1}, 2μgL^{-1}, 20μgL^{-1}) and three levels of salinity (35 ppt, 25 ppt, 17 ppt) were varied, mimicking the timeline of light, moderate and heavy rainfall events. Normal conditions were then slowly re-established over four days to mimic the recovery of the ecosystem post-rain and the experiment continued for a further 7 days to observe potential recovery of the medusae. Pulse-amplitude modulated (PAM) chlorophyll fluorescence, growth and bell contraction rates of medusae were measured. Medusae exposed to the combination of high atrazine and lowest salinity died. After 3 days of exposure, bell contraction rates were reduced by 88% and medusae were 16% smaller in the lowest salinity treatments. By Day 5 of the experiment, all medusae that survived the initial pulse event began to recover quickly. Although atrazine decreased YII under normal salinity conditions, YII was further reduced when medusae were exposed to both low salinity and atrazine simultaneously. Atrazine breakdown products were more concentrated in jellyfish tissues than atrazine at the end of the experiment, suggesting that although bioaccumulation occurred, atrazine was metabolised. Our results suggest that reduced salinity may increase the susceptibility of medusae to herbicide exposure during heavy rainfall events.

1. Introduction

Marine biota are under increasing pressure from a suite of anthropogenic activities and climate change (Crain et al., 2008; Dawson et al., 2011). To accurately assess how marine biota respond to environmental stress, however, experiments need to mimic the conditions biota experience in the field. These conditions include using environmentally relevant levels of the stressors, ensuring that the duration of exposure to the stressor is realistic and (Hughes and Connell, 1999; Macinnis-Ng and Ralph, 2004), if appropriate, examining the effects of simultaneous exposure to multiple stressors that may impart effects that differ from those when biota are exposed to each stressor individually (Folt et al., 1999).

Jellyfish populations have increased in some regions of the world (but decreases or no changes have been reported in others) (Condon et al., 2013), and in some cases have caused severe socio-economic impacts on tourism, fisheries and power industries (Purcell et al., 2007). Some researchers have suggested that jellyfish populations have increased because jellyfish can tolerate many environmental stressors, including pollutants (Lucas, 2001; Templeman and Kingsford, 2010; Gershwin, 2013). The few studies of the effects of pollutants on jellyfish, however, demonstrate that chemical pollutants can exert deleterious effects on jellyfish. For example, exposure to crude oil extract caused 100% mortality of *Pelagia noctiluca* at concentrations ≥20μgL^{-1} after 16 h

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exposure, and survival of ephyrae and larvae decreased with increasing concentrations and exposure time (Almeda et al., 2013). Pollutants such as heavy metals can also bioaccumulate within jellyfish tissues (Templeman and Kingsford, 2010; Almeda et al., 2013; Templeman and Kingsford, 2015), but the sub-lethal effects of these metals have not been tested. Observations that toxic pollutants can negatively affect several life history stages suggest that jellyfish may not be immune to the effects of pollutants in coastal waters.

Like corals, jellyfish from the genus *Cassiopea* harbor zooxanthellae. Zooxanthellae, the microalgal symbionts of corals, jellyfish and other marine invertebrates contribute carbohydrates and other photosynthetic products to their host animals. Obstruction of electron transport through photosystem II (PSII), which occurs as a result of exposure to herbicides, can, therefore, reduce rates of growth and reproduction of the host. In some cases zooxanthellae may be expelled from the host (Jones and Kerswell, 2003; Negri et al., 2005; Cantin et al., 2007). Consequently animals, as well as plants, can be affected by exposure to PSII herbicides. PSII herbicides include, amongst others, atrazine, simazine, ametryn, hexazinone, diuron and tebuthiuron.

Worldwide, herbicides are usually detected in coastal waters in the ngL^{-1} range (Okamura et al., 2003, Shaw et al., 2010) but during heavy rainfall and floods, herbicides may occur in the $\mu\mathrm{gL}^{-1}$ range (McMahon et al., 2005; Davis et al., 2012; Smith et al., 2012), which greatly exceed ecological guideline trigger values that are known to have deleterious effects on marine organisms (ANZECC and ARMCANZ, 2000). Floods, however, are episodic events and elevated concentrations of herbicides generally persist for only a few days (Solomon et al., 1996). Organisms that are adversely affected by the initial exposure to herbicides may begin to recover once concentrations of herbicides decline and the organism begins to metabolise the toxins into other forms. These metabolic breakdown products can occur in high concentrations in coastal environments and, because they are structurally similar to their parent compound, they can also exert deleterious effects on marine organisms (Stratton, 1984; Topp et al., 2000). Despite their persistence in coastal environments, most studies of herbicide exposure have focused only on the effects of the parent compound.

Most studies of the effects of pollutants delivered in heavy rainfall events investigate the effects of continuous exposure over several hours (but see, Macinnis-Ng and Ralph, 2004; Vallotton et al., 2008). Freshwater inflow from heavy rainfall events exposes biota to initially high concentrations of herbicides, but dilution (due to tidal movements) causes herbicide concentrations to decline over several days. Furthermore, several studies have highlighted the importance of investigating the recovery of marine organisms after pulsed exposures to determine the effects of repeated exposures (Solomon et al., 1996; Macinnis-Ng and Ralph, 2004). Macinnis-Ng and Ralph (2004) investigated the effect of two 10 h pulse exposures to copper and the herbicide, Irgarol 1051 on the seagrass *Zostera capricorni* and reported that although a 4-day recovery period between exposures allowed for the recovery of photosynthetic efficiency and chlorophyll concentrations, seagrasses were more vulnerable to the second exposure period. Studies that mimic the variation in herbicide concentrations that occur during rainfall events, and also allow organisms a period of recovery following the return to ambient conditions, may give a more realistic understanding of the long-term effects of herbicides.

During floods, coastal organisms are exposed to multiple stressors simultaneously, including sudden changes in salinity, exposure to pollutants and increased turbidity. Only one study, however, has examined the interactive effects of herbicides and other flood stressors on a cnidarian. That study, which was done on coral and tested a mild 8 ppt reduction in salinity, reported that photosynthetic efficiency was reduced when exposed to Diuron (at 1 and $3\mu\mathrm{gL}^{-1}$) after 10 h incubations, but no significant interaction was detected with reduced salinity (Jones et al., 2003). The salinity reduction tested was small (probably reflecting changes in salinity that occur on coastal coral reefs after heavy rain), and it is likely that larger fluctuations in salinity occur within estuarine ecosystems, which could further exacerbate the effects of herbicides. Studies that consider the effects of multiple stressors and exposure regimes specific to the area of interest are more likely to provide a realistic understanding of how biota are likely to respond to changing environmental conditions.

Jellyfish from the genus *Cassiopea* inhabit shallow areas and so are periodically subjected to heavy rainfall events in which they are exposed to multiple stressors simultaneously. *Cassiopea* sp. rest upside down on the benthos with their oral arms facing upwards to expose their zooxanthellae to light (Hofmann et al., 1996). *Cassiopea* sp. occur in tropical and sub-tropical coastal waters worldwide and can form conspicuous blooms, which are sometimes problematic (Arai, 2001; Mills, 2001).

The objective of this study was to mimic mild to heavy rainfall events by exposing *Cassiopea* sp. medusae to a pulse exposure of reduced salinity and increased concentrations of the herbicide atrazine, followed by a period where salinity and atrazine concentrations returned to ambient conditions. Atrazine was chosen as a model PSII herbicide because it is considered to be one of the five priority photosystem II inhibitor herbicides by the Queensland Government, Australia (Smith et al., 2012). Since the ban of diuron in Australia (in late 2011) (APVMA, 2011), atrazine is the most frequently detected herbicide along the Queensland coastline. We, therefore, selected atrazine as a herbicide of concern for our chosen study ecosystem. We hypothesised that *Cassiopea* sp. medusae exposed to low salinity and atrazine separately would exhibit negative effects on rates of growth, bell contractions and photosynthetic efficiency, but when exposed to both low salinity and atrazine simultaneously the effects would be compounded. At the end of the experiment, concentrations of atrazine and its breakdown products in the tissues of the jellyfish were analysed to determine whether they accumulated and persisted within the tissues.

2. Materials and methods

2.1. Experimental approach

Juvenile *Cassiopea* sp. medusae (size: 19.6 mm ± 0.42 (mean ± 1SE)) were sampled from an enclosed shallow lagoon in Crab Island, Moreton Bay (27.34°S; 153.40°E), Queensland, in March 2014. Atrazine was not detected in water sampled adjacent to the mainland in western Moreton Bay and because Crab Island is on the eastern side of Moreton Bay and distant from any source of agriculture or human settlement, atrazine concentrations at Crab Island were also considered to be negligible. Consequently, the medusae used in the experiment had no prior exposure to atrazine or its breakdown products. Medusae were acclimated to laboratory conditions for 4 weeks and fed newly hatched *Artemia* sp. nauplii daily. Medusae were exposed to 14 h of light per day to accurately mimic diurnal patterns. Aquarium lights were used to imitate the natural solar spectrum, with the wave crest located in the blue spectrum (400–500 nm) to optimise photosynthesis of zooxanthellae.

The full factorial design consisted of two orthogonal factors: salinity (three levels: high salinity (35 ppt, ambient), moderate salinity (25 ppt), low salinity (17 ppt)) and the herbicide atrazine (four nominal levels: no atrazine ($0\ \mu\mathrm{L}^{-1}$), background atrazine ($0.01\ \mu\mathrm{L}^{-1}$), low atrazine ($2\mu\mathrm{gL}^{-1}$), high atrazine ($20\mu\mathrm{gL}^{-1}$)). Salinity