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Chronic effects of atrazine exposure and recovery in freshwater benthic diatoms from two communities with different pollution histories

Rebecca J. Wood[a,*], Simon M. Mitrovic[a], Richard P. Lim[a], Ben J. Kefford[b]

[a] Freshwater and Estuarine Research Group, Ecosystem Security Team, School of Life Sciences, University of Technology Sydney, PO Box 123, Broadway, NSW 2000, Australia

[b] Institute for Applied Ecology, University of Canberra, Canberra, ACT 2601, Australia

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ABSTRACT

Diffuse agricultural runoff into rivers can result in contamination with herbicides for prolonged periods of time. Chronic exposure to herbicides has the potential to alter toxic impacts in primary producers such as benthic diatoms. Determining how individual diatom taxa respond to herbicide exposure over varied exposure durations is essential for assessing herbicide impacts. This study investigated the responses of various benthic diatom taxa and effects at the community level over 12 days of atrazine exposure. Diatom communities were collected from two sites with differing exposure histories; a relatively unpolluted site (Alligator Creek) and an agricultural stream (Barratta Creek) known to be polluted by atrazine and other herbicides. Diatom community composition and the proportion of healthy cells per taxon were assessed at 0, 2, 3, 6, 9 and 12 days of atrazine exposure. Pollution history altered the response of the diatom community to atrazine exposure. In the Alligator Creek diatom community there was a shift in composition towards more tolerant taxa and the loss of sensitive taxa in atrazine exposed treatments. The sensitive taxon (*Gomphonema truncatum*) was consistently affected by atrazine toxicity. Conversely, the polluted Barratta Creek diatom community was not strongly affected by atrazine exposure. Our study shows that during chronic atrazine exposure some taxa demonstrated the ability to recover despite initial toxicity response. Recovery could be an important trait for understanding the ecological effect of herbicide exposure on diatom species in nature and in applied circumstances such as biomonitoring indices.

1. Introduction

The contamination of aquatic ecosystems with herbicides is a major issue of concern in agricultural regions worldwide. Herbicide pollution in rivers often occurs when high concentration pulses enter waterways from agricultural runoff coinciding with high rainfall events (Solomon et al., 1996). However, in the highly polluted Barratta Creek peak concentrations of atrazine (12.3 μg L^{-1}) occurred during periods of low flow coinciding with the end of the sugar cane harvesting period and elevated concentrations of herbicides continued for several continuous months of the year (O'Brien et al., 2016). Additionally, low-level herbicide concentrations have been detected year round in other rivers (Shaw et al., 2010; Smith et al., 2012). As a result, freshwater organisms are likely to be exposed to herbicides under both acute and chronic exposure scenarios (Dorigo et al., 2004; Smith et al., 2012). Both short and long term herbicide exposure have the potential to alter the structure and function of primary producers such as benthic diatom communities (Larras et al., 2012; Magnusson et al., 2008, 2012; Ricart et al., 2009; Rimet and Bouchez, 2011). In order to assess potential herbicide impacts it is essential to investigate the responses of individual diatom taxa as well as the community to herbicides over a range of exposure durations.

Exposure to herbicides over different durations will likely alter the potential physiological and ecological effects to the benthic diatom community (Gustavson et al., 2003). Under short-term exposure (defined here as ≤ 96 h) herbicide toxicity has been shown to alter photosynthesis (Magnusson et al., 2010), growth (Larras et al., 2012) and cell health (Wood et al., 2014, 2016a,b) of benthic diatoms. Whereas, longer exposure of diatoms to herbicides can result in increased herbicide tolerance through physiological acclimation (Roubeix et al., 2011; Tiam et al., 2015) and taxa that are initially impaired may have the ability to recover and subsequently outcompete others that are slower to, or cannot, recover (Carder and Hoagland, 1998; Magnusson et al., 2008, 2012). Alternatively, cells which do not develop tolerance to herbicide exposure can become affected with subsequent exposure as stress from the herbicide builds up (Nelson et al., 1999). Freshwater benthic diatoms have doubling rates of approximately 0.1–2.2 d^{-1} (Admiraal, 1976; Gould and Gallagher, 1990) and longer exposure

* Corresponding author:
E-mail address: Wood.Rebecca.Jane@gmail.com (R.J. Wood).

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periods will involve multi-generational exposure, which can increase toxicity (Kefford et al., 2008; Rose et al., 2002). Multigenerational exposure can also lead to the selection of better adapted individuals, resulting in genetic adaptation, which leads to increased tolerance and in turn resistance (Stachowski-Haberkorn et al., 2013). At the community level, herbicide exposure can exert a selective pressure that results in the dominance of more tolerant taxa to the detriment of sensitive taxa (Blanck, 2002). This compositional shift can alter sensitivity at the community level resulting in increased pollution tolerant communities (Magnusson et al., 2012; Pesce et al., 2010; Tlili et al., 2011). Establishing how herbicide exposure duration affects freshwater diatoms and whether the same taxa that are sensitive to short-term exposure are also similarly affected by longer exposure durations is critical in understanding the effects of herbicides on the benthic diatom community.

The current study investigated the response of freshwater benthic diatoms within natural diatom communities to the photosystem II (PSII) inhibiting herbicide atrazine, over 12 day continuous exposure laboratory experiments. Diatom communities were collected from two locations with differing pollution histories; Alligator Creek, a relatively unpolluted reference site, and Barratta Creek, an agriculturally impacted stream known to be polluted by herbicides, including atrazine and other PSII herbicides (Davis et al., 2008 O'Brien et al., 2016). Within these benthic communities the number of healthy diatom cells per taxon was assessed on day 0 (prior to exposure), 2, 3, 6, 9 and 12 of atrazine exposure. The aim was to determine whether exposure duration alters the effect of atrazine on specific diatom taxa and to identify taxa capable of recovery in the presence of atrazine. Additionally, we assessed chronic effects at the community level using changes in species composition and relative abundance.

2. Materials and methods

2.1. Study sites and diatom collection

Benthic diatoms were collected from Alligator Creek and Barratta Creek on the 24th of October 2012. These rivers flow into the Great Barrier Reef Marine Park (GBRMP), a World Heritage listed area. The GBRMP catchment covers 424,000 km^2 and includes 35 smaller coastal catchments of which these rivers are included. The collection sites are located in the dry tropical climatic region approximately 30 km (Alligator) and 70 km (Barratta) south west of Townsville, Queensland, Australia.

Alligator Creek originates in Bowling Green Bay National Park and flows through the Ramsar listed wetland, Bowling Green Bay (ANCA, 2001). Diatom samples were collected from a sampling site (19°25.777′S, 146°56.599′E) in the upper catchment of the river at the base of the National Park, with no agricultural activity upstream. The river is approximately 8 m wide with mostly large cobbles, boulders and bedrock substrate. The upstream and surrounding vegetation is dense eucalypt forest and rainforest. There is only recreational activities upstream of the sampling location, therefore herbicide impacts at the collection site are considered negligible (Lewis et al., 2009).

Barratta Creek is located in the lower Burdekin River region, an agricultural district supporting extensive sugarcane farming (Davis et al., 2008). Barratta Creek also drains into Bowling Green Bay, a wetland listed in Australia's National Directory of Important Wetlands and a Ramsar wetland of international significance (ANCA, 2001). The collection location (19°42.416′S, 147°08.850′E) is situated in the upper Barratta Creek catchment, with predominantly agricultural land uses upstream (grazing, mixed horticulture and sugarcane) (Davis et al., 2008). The collection site has a narrow sparsely vegetated corridor of small eucalypt trees and grasses on either side of the approximately 5 m wide river channel comprising a rocky substrate with gravel and sand embankments. This location is highly impacted by agricultural herbicides such as atrazine, diuron, hexazinone 2,4-D and MCPA, which are used in the sugarcane industry (Davis et al., 2008; Davis et al., 2012). Concentrations of the PSII herbicides, atrazine and diuron exceeded the ecological protection guidelines for several continuous months of the year, with maximum recorded concentrations of 12.3 μg L^{-1} atrazine and 12.8 μg L^{-1} diuron (O'Brien et al., 2016). Mixtures of multiple PSII herbicides are frequently detected at Barratta Creek and their toxic effects to primary producers such as benthic diatoms have been shown to be additive (Magnusson et al., 2010). The mixture toxicity of PSII herbicides has been estimated by calculating the toxic equivalency quotient (TEQ) which adds the concentrations of PSII inhibitors in a mixture after applying a toxic equivalency factor (TEF) based on the response of the freshwater alga *Chlorella pyrenoidosa* (TEQ_{CP}). The estimated toxicity of PSII herbicide mixtures at Barratta Creek has exceeded the atrazine trigger value for ecological protection (13 μg L^{-1}) for 30 consecutive days (Smith et al., 2012) with a maximum TEQ_{CP} atrazine equivalent concentration of 807 μg L^{-1}.

The benthic diatoms were collected by scrubbing pebbles and cobbles from the bottom of the riverbed with a soft bristled toothbrush to remove the attached diatoms. Rocky substrates were sampled from various locations within an approximately 20 m reach of river including riffles, pools and edge zones. The detached benthic diatoms were washed into trays and pooled into a composite sample per site. These samples were stored in the dark and transported directly to the laboratory at the same temperature as that of the site water. Water quality conditions at the time of diatom collection are summarised in Supplementary Table S1 and published information is available for Barratta Creek coinciding with our study from that of O'Brien et al. (2016) and its previous condition from Davis et al. (2008) and for Alligator Creek (Lewis et al., 2009).

2.2. Determination of atrazine concentrations at the sampling locations

Grab water samples were taken at each of the collection sites at the time of diatom collection. The water was collected into solvent rinsed, 1 L amber glass bottles, transported on ice and placed in a freezer overnight in the dark at 4 °C before being sent for measurement of atrazine concentrations by liquid chromatography tandem mass spectrometry (LC–MS/MS) at Eurofins Agroscience Testing Pty Ltd.

2.3. Toxicity tests

The collecting, transport and toxicity testing of benthic diatoms followed the method described in Wood et al. (2014), except that the test used in the current study was over a longer duration (12 days rather than 2 days). The live benthic diatom samples were allowed to acclimatise to conditions of the temperature-controlled laboratory for one hour before commencement of the toxicity tests, which commenced within 3 h of diatom collection from the field. Two toxicity tests were conducted simultaneously with diatoms collected from each site under the same experimental conditions. The toxicity tests were conducted over 12 days at 24 °C under a light intensity of 100 μmol m^{-2} s^{-1} on a 12 h light/dark cycle. For the test treatments 1 mL subsamples of the benthic diatoms were pipetted into 30 mL test vials with river water from the corresponding site to a final volume of 10 mL. Herbicide exposure treatments were spiked with atrazine at predetermined nominal concentrations of 50, 200 and 500 μg L^{-1}. These concentrations were shown to elicit a response in the sensitive taxa (Wood et al., 2014; Wood et al., 2016a; b) and despite being higher than the measured peak concentrations of atrazine recorded at Barratta Creek (12.3 μg L^{-1}), correspond with estimated mixture toxicities of PSII herbicides frequently detected in the study region (Smith et al., 2012). Atrazine stock solutions were prepared by dissolving analytical grade atrazine (Pestanal) in 10 mL 99% ethanol. Two control treatments were prepared for each site (no atrazine) that is river water controls (using water from the respective collection sites) and carrier controls with 0.05% ethanol in site water (final ethanol concentration equal to the maximum concentration in the atrazine treatments). All treatments were replicated