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# Determining the relative sensitivity of benthic diatoms to atrazine using rapid toxicity testing: A novel method

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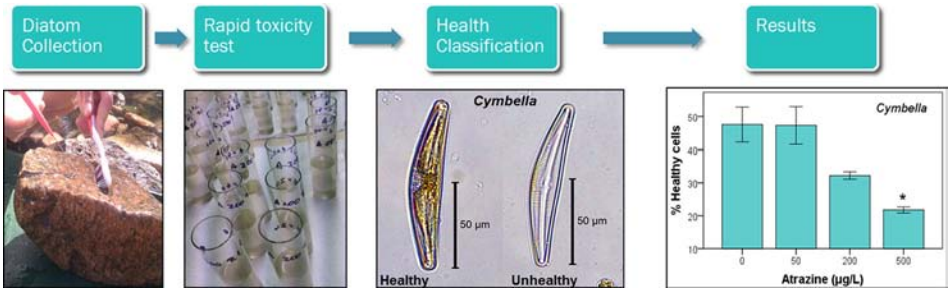
## HIGHLIGHTS

- A novel method is described for the rapid toxicity testing of benthic diatoms to herbicides.
- Atrazine sensitivity is determined for individual diatom taxa from a natural benthic community.
- Atrazine concentration had a negative effect on the health of diatom cells in the sensitive taxa.
- Effects on community structure were evident after 48 h of atrazine exposure.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Herbicides pose a potential threat to aquatic ecosystems, especially to phototrophic organisms such as benthic diatoms. Benthic diatoms may be a valuable indicator of the toxic impacts of herbicides in aquatic systems. However, this requires information on the herbicide sensitivity of a wide range of freshwater benthic diatom taxa. Unfortunately this information is only available for a limited number of species as current methods of developing new algae toxicity tests on individual taxa are lengthy and costly. To address this issue, we developed a new rapid toxicity test method to test natural benthic communities, from which the relative herbicide sensitivity of many individual taxa can be derived. This involved the collection of natural benthic communities from rocks *in situ*, which were placed directly into laboratory toxicity tests. Sensitivity data for several diatom genera in a 48 hour exposure toxicity test were produced, without the need for cultures or multiple site visits. After exposure to the highest treatment of atrazine (500 µg $L^{-1}$) there were significant declines of healthy cells in the most sensitive genera: *Gomphonema* declined by 74%, *Amphora* by 62%, *Cymbella* by 54% and *Ulnaria* by 34% compared to control levels. In contrast, the genera, *Eunotia*, *Achnanthidium* and *Navicula*, had no statistically significant decline in cell health. This method can identify the diatom taxa most at risk of herbicide toxicity within the natural benthic diatom community. The rapid toxicity testing method presented is a simple and effective method to obtain sensitivity data for multiple taxa within a natural benthic diatom community in a relatively short period of time.


## 1. Introduction

Herbicide contamination of freshwater ecosystems poses a potential threat to primary producers, such as benthic diatoms, and they may be a valuable indicator community for toxic impacts (DeLorenzo et al., 2001). Benthic diatoms are ubiquitous and respond rapidly to environmental conditions; therefore, changes in community composition due to herbicide toxicity may reflect past herbicide concentrations (Burns and Ryder, 2001; Villeneuve et al., 2011). Herbicide exposure in streams typically occurs as pulses associated with diffuse agricultural runoff, and as a result, routine (i.e. calendar based) sampling of herbicides will most likely underestimate herbicide concentration and thus toxicity (Davis

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et al., in press). In order to address this, chemical monitoring needs to include event based sampling after rainfall and during floods to estimate the peak concentration of herbicides and/or include the use of passive samplers to estimate the average concentration. However, these measures require multiple site visits, increasing the cost of monitoring. Furthermore, with any chemical monitoring there is uncertainty as to the ecological risk of the chemicals observed and the chemicals detected may not be the entire suite of chemicals present in the field (Magnusson et al., 2008). Consequently, there is a need for biomonitoring tools that give an integrated response to chemicals over time, and freshwater benthic diatoms may be a cost effective and ecologically relevant solution for herbicides (Debenest et al., 2009; Morin et al., 2009).

Linking field effects to any one particular stressor in the environment can be problematic due to the range of variables that can alter community structure and the influence of multiple stressors (Morin et al., 2009; Schäfer et al., 2007). However, Schäfer et al. (2011a) proposed a conceptual model for trait based biomonitoring indices that link exposure to a specific stressor with community composition changes in the field, such as the SPEcies At Risk (SPEAR) index (Liess and Ohe, 2005). The $SPEAR_{pesticides}$ index has been developed using macroinvertebrates to describe changes in the proportion of sensitive taxa within a community, relative to the intensity of pesticide stress (Liess and Ohe, 2005). The key trait used in $SPEAR_{pesticides}$ is the sensitivity of macroinvertebrate taxa to organic toxicants (Liess and Ohe, 2005; Schäfer et al., 2007). $SPEAR_{pesicides}$ has been used successfully in Europe and also in Southeast Australia, to link pesticide exposure (mostly insecticides and fungicides) to field effects (Liess et al., 2008; Schäfer et al., 2011b). However, $SPEAR_{pesticides}$ is less effective at predicting herbicide toxicity as it uses macroinvertebrates as indicators which respond more strongly to insecticides and fungicides (Schäfer et al., 2011c). Benthic diatoms may be a more suitable indicator community to assess herbicide toxicity, especially photosystem II inhibitors (PSII), as their phytotoxic effects have been established (Debenest et al., 2010; Magnusson et al., 2010, 2012).

The principle impediment to developing a biomonitoring index for herbicides, based on the community composition of diatoms (or other primary producers) is lack of information on how particular taxa respond to herbicides (Culp et al., 2011; Morin et al., 2009; Roubeix et al., 2011). Although some information exists on the toxicity of herbicides to a few freshwater benthic diatom species (Debenest et al., 2009; Larras et al., 2012; Magnusson et al., 2010; Tang et al., 1997), for any particular region, there are very few taxa with herbicide sensitivity data (Magnusson et al., 2012). This is in part due to the time constraints and costs of current standard toxicity tests which involve the use of single species cultures to determine individual sensitivities. Cultures of most species are unavailable and obtaining sensitivity data for numerous species by standard toxicity testing methods would be very time consuming. A new method that can produce sensitivity data for a number of local taxa in a relatively short period of time would be ideal for obtaining the required data for a traits-based monitoring index that can detect herbicide toxicity in rivers (Culp et al., 2011). We followed the rapid toxicity approach which aims to determine herbicide toxicity to multiple taxa from a multispecies community in a relatively short period of time (Hickey et al., 2009; Kefford et al., 2005). Other studies either use single species cultures to produce this sensitivity data for individual taxa (Larras et al., 2013; Magnusson et al., 2010; Roubeix et al., 2011), or use community level measures of health such as photosynthetic inhibition that cannot determine which taxa within the community are contributing to the sensitivity (Magnusson et al., 2012; Proia et al., 2011; Prosser et al., 2013).

This paper establishes a new method to determine the relative herbicide sensitivity of field derived freshwater benthic diatom taxa using rapid toxicity tests. These tests aim to produce relative sensitivity data for several freshwater diatom taxa in one 48 hour test (see Kefford et al., 2003). The current study utilises a new approach to place benthic diatoms collected *in situ* directly into rapid toxicity tests that can determine the relative sensitivity of the individual diatom taxa from within the freshwater benthic community.

## 2. Materials and methods

### 2.1. Diatom collection locations

Diatoms were collected from Bluewater Creek (−19.14385, 146.26817) on the 18th of May 2012. The creek is located in North Queensland, Australia, at the base of Paluma State Forest near the town of Bluewater and is surrounded by eucalypt woodland. The stream substrate at the sample site is mostly large boulders, cobbles and pebbles, with a mean channel width of 7 m and highly diverse habitats present including deep and shallow pools, falls, runs and shallow riffles. The study site was chosen as there is no agriculture and only recreational activities occurring upstream of the site. The site is therefore considered a reference site for agricultural impacts such as herbicide pollution.

### 2.2. Sampling of natural benthic diatom communities

Pebbles and cobbles (approximately 5–25 cm in the longest axis) from the stream bed were chosen at random from various areas of a 50 m section of the stream bed and placed in trays for scrubbing. Multiple areas within a 50 m stretch of stream bed were sampled in order to include a variety of habitat types; riffles, pools and falls, for the purpose of obtaining the greatest possible number of taxa in a composite site collection. Areas which were stagnant pools and also very shallow areas likely to have been recently dried out were avoided to minimise collection of dead material. The benthic diatoms were removed from the rocks by scrubbing with a soft bristle toothbrush, using a squirt bottle with site water to wash off the detached material into a collection tray. The detached benthic diatoms were collected into a 500 mL plastic sample container as a composite sample, which was stored in the dark at site water temperature (21 ± 1 °C) for transportation to the lab.

### 2.3. Rapid toxicity tests

The benthic diatoms were exposed over 48 h to atrazine to determine the relative sensitivities of the taxa within the community. Tests were conducted in a controlled temperature laboratory at 24 ± 2 °C at a light intensity of 20 μmol $m^{-2}$ $s^{-1}$ (±10%), under a 12:12 hour light:dark cycle. After transportation to the lab the experiment was initiated within 4 h of sampling and included a 1 hour acclimatisation period to stabilise the temperature to that of the room.

The solution containing the removed benthic diatom community from Bluewater Creek was homogenised by gentle shaking and divided into 1 mL aliquots randomly assigned to 18 × 40 mL test vials by pipette. The test vials were then made up to a final volume of 20 mL with site water and spiked with a known atrazine herbicide concentration depending on treatment. The atrazine stock solution was prepared by dissolving analytical grade atrazine (Sigma Aldrich, CAS 1912-24-9) in site water using a carrier of 99% ethanol to increase the solubility of atrazine (2% *v:v*) with the maximum final volume of ethanol in the treatments being 0.05% (Magnusson et al., 2010). An ethanol control treatment with a final volume of 0.05% ethanol was included and compared to a site water only control after 48 h to eliminate carrier effects. All herbicide treatments were compared to the ethanol control. An additional control treatment at the start of the experiment ($t = 0$) was also prepared to indicate the diatom community and health at the start of the experiment. The experiment had a static water supply, without renewal of water or agitation for the duration of the test period as is common in algal bioassays (Larras et al., 2012; Magnusson et al., 2008). Diatoms were exposed to atrazine concentrations of 50, 200 and 500 μg $L^{-1}$, which were shown to elicit a response in the sensitive taxa from trial tests (data not shown) and are environmentally realistic in the region (Smith et al., 2012). All treatments and controls were replicated thrice.