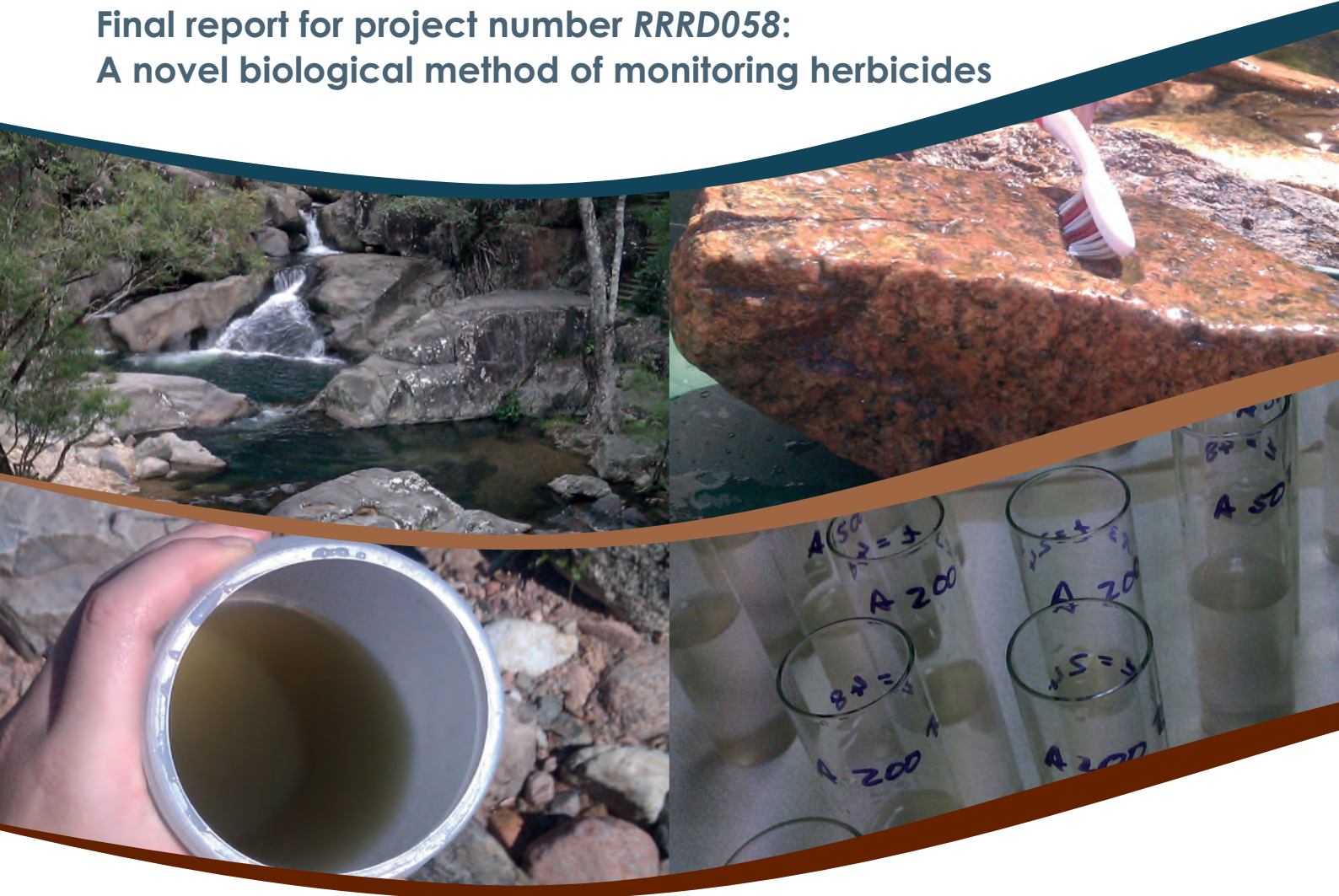


Reef Rescue Research & Development

Technical Report

Biomonitoring effects of pesticides in rivers
draining on to the Great Barrier Reef
Final report for project number *RRRD058*:
A novel biological method of monitoring herbicides



Ben J Kefford, Rebecca J Wood, Simon Mitrovic and Peter von der Ohe



Biomonitoring effects of pesticides in rivers draining on to the Great Barrier Reef (Project RRRD058)

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Acronyms Used In This Report

DSITIA	Department of Science, Information Technology, Innovation and the Arts (Queensland)
GBR	Great Barrier Reef
GBRWHA	Great Barrier Reef World Heritage Area
GC-MS	gas chromatography-mass spectrometry (a method of chemical analysis)
HLCMS	high performance liquid chromatography–mass spectrometry (a method of chemical analysis)
PSII	photosystem II inhibitors (a group of herbicides)
SPEAR	SPEcies At Risk (a classification of taxa)
SPEnotAR	SPEcies not At Risk (a classification of taxa)
SPEAR_{herbicides}	SPEcies At Risk from herbicides (a diatom-based biomonitoring index)
SPEAR_{pesticides}	SPEcies At Risk from pesticides (a macroinvertebrate-based biomonitoring index) [#]
SPEAR_{salinity}	SPEcies At Risk from salinity (a macroinvertebrate based biomonitoring index)
TEQ_{cp}	atrazine Toxic Equivalent concentrations for <i>Chlorella pyrenoidosa</i> (an index of relative toxicity of multiple PSII herbicides)
TEQ_{so}	atrazine Toxic Equivalent concentrations for <i>Scenedesmus obliquus</i> (an index of relative toxicity of multiple PSII herbicides)
TU_{sc}	Toxic units <i>Selenastrum capricornutum</i> (an index of relative toxicity of all pesticides)
TU_{DM}	Toxic units <i>Daphnia magna</i> (an index of relative toxicity of all pesticides)

[#] In this report we will retain the use of SPEAR_{pesticides} to be consistent with usage of the term in other documents, however we note that being macroinvertebrate-based SPEAR_{pesticides} responds most strongly to insecticides and fungicides and only responds to herbicides if they are at very high concentrations.

Abbreviations Used In This Report

biomonitoring	biological monitoring
log	logarithm
max	maximum

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Executive Summary

Coral cover across large parts of the Great Barrier Reef (GBR) in north Queensland, Australia has declined since the 1960's, with the exception of the northern section, where little intensive agriculture takes place in the respective river catchments draining in the Great Barrier Reef. Agricultural runoff - containing nutrients, sediments and pesticides - that reaches the marine environment via rivers is believed to be a significant factor in this decline. Agricultural runoff is also believed to be an important stressor for other estuarine and marine ecosystems within the GBR world heritage area (GBRWHA), including seagrass and mangrove systems. Of the pesticides in agricultural runoff, persistent herbicides, particularly the photosystem II inhibitors (hereafter PSII), are believed to pose the greatest risk to ecosystems and organisms in the GBRWHA.

While it will not replace chemical monitoring, biological monitoring (biomonitoring) of effects of pesticides on biotic communities can complement existing chemical monitoring programs and together both methods can provide a more comprehensive picture of the state of environmental contamination. Aquatic organisms live continuously in the water and are therefore exposed to all chemicals in the water over their lifetime in contrast to grab samples that quantify selected chemicals in a sample at an instant in time. So biomonitoring studies can assure that the effects of peak concentrations of pesticides are captured and that no ecologically relevant chemicals in the water are overlooked due to low concentrations or lack of available testing method. There is uncertainty as to the ecological relevance of concentrations of many pesticides and much uncertainty as to the effects of mixtures of multiple pesticides. Biomonitoring by measuring the ecological effects, and not concentrations of a potentially incomplete set of chemicals, side-steps these areas of uncertainty. Biomonitoring detects the effect of the pesticides and if an effect is detected it indicates ecologically relevant exposure (although not which particular chemical or mixture caused the effect). Biomonitoring also allows, at relatively modest cost, greater spatial coverage than current chemical monitoring programs. Finally, biomonitoring allows for the re-analysis of historical biotic data, which in the case of rivers draining into the GBR, allows the historical trends to be considered, as some biotic data precede chemical monitoring.

This document reports on the findings of a study that developed biomonitoring methods to detect the impacts of pesticides on freshwater organisms in rivers draining into the GBRWHA. In particular, we modified an existing stream macroinvertebrate-based SPEcies At Risk from pesticides (SPEAR_{pesticides}) index, that has up until now been used in regions with temperate climates, so that it is suitable for use in tropical north Queensland. This index has been successfully used across Europe and in Victoria, Australia and responds specifically to impacts on macroinvertebrate communities as a result of pesticides, but appears to be largely unaffected by other environmental factors. As macroinvertebrates are not photosynthetic organisms they are generally more tolerant of direct effects of herbicides compared to algae and plants, and thus SPEAR_{pesticides} largely detects impacts from insecticides and fungicides.

To detect impacts of herbicides, we devised a new biomonitoring index based on freshwater benthic diatoms (a common group of micro photosynthetic algae with a cell wall made of silica) to detect impacts of herbicides. This new index, called SPEAR_{herbicides}, is based on similar principles as the macroinvertebrate-based SPEAR_{pesticides}. (Note that we retain the

name SPEAR_{pesticides} for the macroinvertebrate-based index, despite the fact that herbicides are pesticides, to be consistent with the usage of the term SPEAR_{pesticides} elsewhere).

The SPEAR_{herbicides} index was developed by conducting a series of rapid toxicity tests designed to test the sensitivity of large number of diatom taxa collected from rivers draining into the GBR. These rapid tests were specifically developed as part of the current study. The results of these toxicity tests were used to classify diatom taxa as either SPEcies At Risk (SPEAR) or SPEcies not At Risk (SPEnotAR) from herbicides. For species which we did not test, their classification was based on extrapolation from related species of the same genus. Genera that were untested were unclassified and not used in the calculation of SPEAR_{herbicides} that appears in this report. From this classification, the final SPEAR_{herbicides} index was derived. The rapid tests included, in addition to herbicide concentration, experimentally manipulated up to 8 types of herbicide, light intensity, nutrient levels and source of diatoms. Results showed that the relative sensitivity of diatom genera was independent of herbicide type, light intensity, nutrient level and collection sites.

We sampled the stream macroinvertebrate and benthic diatom communities at the 11 Department of Science, Information Technology, Innovation and the Arts (DSITIA) pesticide monitoring sites that are part of the Great Barrier Reef Catchment Loads Monitoring Program and at 3 reference sites in rivers before and after the 2011/12 and 2012/13 wet-seasons. At present we have received pesticide concentration data from the 2011/12 wet-season but not the 2012/13 wet-season. When the data from the 2012/13 wet-season are available, analysis of the biotic data from the season will occur.

After the 2011/12 wet-season the SPEAR_{herbicides} at the pesticide monitoring sites was inversely correlated with the estimates of the peak concentration of photosynthesis II inhibiting herbicides as expressed as atrazine Toxic Equivalent concentrations for *Scenedesmus obliquus* (TEQso) during that wet-season. This indicated that the relative abundance of herbicide sensitive diatom species declined with increasing herbicide concentration. This result suggest that the chemical monitoring program is not missing ecologically significant peak concentrations, un-measured chemicals nor unexpected effects of mixtures of chemicals. If the relationship is confirmed in the 2011/13 wet-season, it would strengthen the case that the developed SPEAR_{herbicides} index can detect the effects of herbicides on diatom communities.

The relative abundance of pesticide sensitive diatom species appeared to recover during the dry-season, so that SPEAR_{herbicides} index prior to the 2011/12 and 2012/13 wet-seasons was not related to TEQso concentrations.

The values of the macroinvertebrate-based SPEAR_{pesticides} index suggested that these chemicals are generally no major problem to invertebrates at the sites sampled in 2011/12. Furthermore the values of the SPEAR_{pesticides} index suggest that there are no ecologically relevant (for invertebrates) effects that have been missed by the pesticide monitoring, mixtures of multiple chemicals or un-measured chemicals.

The project has successfully modified the SPEAR_{pesticides} index and developed the new SPEAR_{herbicides} index. They appear to be promising tools to complement chemical monitoring of pesticides in the catchments of the GBR. We recommend that they are used in conjunction with the chemical monitoring program before and after subsequent wet-

seasons. Some further research is also recommended involving mesocosm experiments to be able to prove causality between the indexes and pesticides exposure.

The cost of biomonitoring in conjunction with chemical monitoring of pesticides is extremely cost effective. The collection and processing of diatom and macroinvertebrates samples is approximately \$6,000 per site per year in comparison to some \$85,000 per site per year for chemical monitoring of pesticides (note neither of these estimates include data analysis and reporting costs). So at relatively modest costs additional costs, biomonitoring can add considerable new information as the ecological effects of the pesticides and increased confidence that all ecologically relevant compounds and their peak concentrations are being captured by the chemical monitoring program. Additionally biomonitoring could be used to screen a large number of sites for indications of pesticide toxicity, where chemical monitoring does not occur.

Introduction

The Great Barrier Reef (GBR) is the largest coral reef system in the world and supports incredible biodiversity, recreational and commercial fishing and tourist industries. Since the 1960's coral cover has reduced throughout the Barrier Reef, except in the northern or Cape York section where agriculture runoff is considered minimal. Poor water quality, in particularly elevated nutrients, sediments and pesticides, from agricultural runoff is believed to be a major contribution the decline of coral cover and also poses risk to sea grass and mangrove ecosystems. In terms of pesticides the chief concern for the GBR of pesticides are believed to be herbicides, particularly the photosystem II inhibitors (PSII). This documents reports on a project developing methods for biological monitoring (biomonitoring) of ecological effects of pesticides in rivers draining on to the GBR. In particularly it adapts the existing SPEcies At Risk (SPEAR) from pesticides stream macroinvertebrate biomonitoring index for use in north Queensland and it develops a similar index designed to detect the effect of herbicides.

Given the potential harm that pesticides pose to the GBR and organisms that live on it, it is important to monitor pesticides in rivers which drain into the reef. Such monitoring can determine the risk these chemical pose to the reef, trends in pesticides entering the GBR, determine if pesticide load reduction targets are being met and identify catchments and farming practices responsible for significant inputs.

There are several technical challenges in determining and interpreting data from the sampling of pesticides in rivers. Significant pesticide concentrations in rivers occurring episodically associated with spray drift and rainfall washing pesticides from agricultural fields into aquatic environments. Consequently, it is possible to miss the peak concentrations of pesticides. There are also a large number of pesticides and other chemicals registered for use in Australia and there remains much uncertainty as to their ecological effect of most of these chemicals. This is especially when organisms are exposed to a cocktail to multiple substances, in the natural environment which includes a number of non-chemical stresses (e.g. temperature extremes, floods and droughts). So while chemical monitoring can determine the concentration of compounds that are targeted, there will always remain uncertainty as to the ecological relevance of the concentrations measured.

Biomonitoring of rivers is determining the health of rivers by monitoring the organisms living in them rather than by measuring physicochemical attributes of the water. Biomonitoring of pesticides will never replace chemical monitoring programs. Biomonitoring does, however, offer a number of advantages for complementing chemical monitoring programs of pesticides because it measures the ecological responses of organisms continuously living in the monitored environment. Thus if there are ecological relevant short-term peak concentrations, effects of mixtures of chemical, influence of non-chemical stressors, non-measured chemicals at toxicologically relevant levels etc., then the organisms will respond and biomonitoring can indicate that the health of the river has be adversely affected.

There are also challenges with biomonitoring as organisms respond to many stressors and environmental factors, including non-chemical and specific taxa present vary spatial and temporally even in the absence of any pesticides or other human disturbance. The use of traits (or attributes) of organisms rather than their taxonomic identity has the potential to

reduce this problem as while the taxonomic composition of communities is highly variable their trait composition is more stable. Schäfer et al. (2011a) proposed a conceptual model for trait based biomonitoring. Under this conceptual model; a key trait for any stressor that acts physiologically, such as a chemical toxicant, is the physiological sensitivity of taxa to that stressor. This contrasts with stressors such as mechanical disturbance (flooding) which do not act on the physiological level but which physically wash organisms away or alter habitat. Schäfer et al. (2011a) conceptual model also suggest that for episodic stressors that traits that allow organisms to avoid the stress or rapidly recover should be important. This conceptual model explains why the macroinvertebrate-based SPEAR_{pesticides} and SPEAR_{salinity} indexes respond to pesticide toxicity and salinity, respectively but not other stressors.

SPEAR_{pesticides} (Liess and Von der Ohe 2005, Schäfer et al. 2007) has been used successfully used in Europe and also in Victoria, to link pesticide exposure to field effects (Liess et al. 2008, Schäfer et al. 2011b). However, primary producers such as benthic diatoms, are relatively more sensitive to herbicides than macroinvertebrates, especially on the suite of herbicides known as photosystem II inhibitors, which act on the photosynthetic pathway (DeLorenzo et al. 2001, Debenest et al. 2010). Thus, SPEAR_{pesticides} may be less effective at predicting herbicide toxicity – the main concern for the GBR – as it is correlated with pesticide toxicity (mostly insecticides and fungicides) and not herbicides.

(In this report we will retain the use of SPEAR_{pesticides} to be consistent with usage of the term in other documents. However we note that being macroinvertebrate-based SPEAR_{pesticides} responds most strongly to insecticides and fungicides and only responses to herbicides if they are at very high concentrations.)

Herbicide toxicity poses a threat to the receiving freshwater environments as well as the estuarine and marine ecosystems in the GBR world heritage area (GBRWHA) and tools for the assessment of herbicide toxicity impacts are needed (Brodie et al. 2012). The extensive use of herbicides in agricultural regions of North Queensland has resulted in the widespread contamination of waterways and recent chemical monitoring within the catchments of the GBR has detected herbicide concentrations above the guidelines for ecological protection (Lewis et al. 2009, Smith et al. 2012). The focus of this research is develop new methods for the determination of herbicide sensitivity data to local freshwater benthic diatoms, that can provide a link between contaminant loads and ecological effects (Magnusson et al. 2010). It is our aim that this sensitivity data will be used in the creation of a new biomonitoring index for herbicides, which would be a step towards setting more meaningful management goals and identifying areas of greatest risk of herbicide toxicity (Brodie et al. 2009).

The research described in this report focus around a number of research goals.

1. The development of a method of rapid diatom toxicity tests to estimate the herbicide sensitivity of many field collected diatom species so that we could establish the relative herbicide sensitive of the majority of diatom taxa in rivers draining on to the GBR.

2. Estimate the relative sensitivity of diatoms to various herbicides to determine whether the ranking of herbicide sensitivity of diatoms varies with different types of herbicides.
3. Determine if light intensity during exposure alters the relative sensitivity of diatoms to herbicides.
4. Assign traits relevant for SPEAR_{pesticides} for stream macroinvertebrates known to occur in rivers draining on to the GBR.
5. Classify diatom taxa which occur in rivers draining on to the GBR as at risk or not at risk from herbicides.
6. Determining if the macroinvertebrate-based SPEAR_{pesticides} and the diatom-based SPEAR_{herbicides} are correlated with toxicity of the measured chemicals to model species of invertebrates and plants, respectively.

Chapter 1 - Determining the relative sensitivity of benthic diatoms to atrazine using rapid toxicity testing: a novel method

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, information on the herbicide sensitivity of a wide range of freshwater benthic diatom taxa is required in order to develop biomonitoring tools to assess herbicide toxicity. 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 method to test natural benthic communities in rapid toxicity tests, from which the relative herbicide sensitivity of 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 (500µg/L) 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. Whilst more tolerant genera, Eunotia, Achnanthes and Navicula, had no statistically significant decline in the proportion of healthy cells. 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.

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 et al. 2011). 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 (Schäfer et al. 2007, Morin et al. 2009). However, Schäfer et al. (2011a) proposed a conceptual model for trait based biomonitoring indices that link exposure to a specific stressor with changes in community composition 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_{pesticides} 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. 2011c). 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. 2011b). 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, Magnusson et al. 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 (Morin et al. 2009, Culp et al. 2011, Roubex et al. 2011). Although some information exists on the toxicity of herbicides to a few freshwater benthic diatoms communities (Debenest et al. 2009, Magnusson et al. 2010, Larras et al. 2012), 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 many local 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 (Kefford et al. 2005b, Hickey et al. 2009). Other studies either use single species cultures to produce this sensitivity data for individual taxa (Magnusson et al. 2010, Roubéix et al. 2011, Larras et al. 2013), or use community level measures of health such as photosynthetic inhibition that cannot determine which taxa within the community are contributing to the sensitivity (Proia et al. 2011, Magnusson et al. 2012, 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 (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.

Materials and methods

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 7m and highly diverse habitats present including deep and shallow pools, falls, runs and shallow riffles. The study site was chosen due to there being 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.

Sampling of natural benthic algae communities

Pebbles and cobbles (approximately 5-25cm in longest axis) from the stream bed were chosen at random from various areas of a 50m section of the stream bed and placed in trays for scrubbing. Multiple areas within a 50m 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 500mL plastic sample container as a composite sample, which was stored in the dark at site water temperature ($21 \pm 1^\circ\text{C}$) for transportation to the lab.

Rapid toxicity tests

The benthic diatoms were exposed over 48 hours 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 $20\mu\text{mol}/\text{m}^2/\text{s}$ ($\pm 10\%$), under a 12:12 hour light: dark cycle. After transportation to the lab the experiment was initiated within 4 hours 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 1mL aliquots randomly assigned to 18 x 40mL test vials by pipette. The test vials were then made up to a final volume of 20mL with site water and spiked with a known atrazine herbicide concentration depending on treatment. The atrazine stock solution was prepared 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 hours to eliminate solvent effects. All herbicide treatments were compared to the ethanol controls; which shall be termed 'controls' hereafter. There were 3 herbicide exposure treatments, consisting of atrazine concentrations of 50, 200 and 500 $\mu\text{g}/\text{L}$, which were shown to elicit a response in the sensitive taxa from trial tests (data not shown). 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. All treatments and controls were replicated thrice. Three additional replicates were prepared at the start of the experiment in the same manner as each herbicide test treatment (50, 200, 500 $\mu\text{g}/\text{L}$) to be analysed for determination of the actual atrazine concentrations, which were within 15% of the nominal values (data not shown).

Preservation of samples

After the exposure period (48 hours) the contents of each replicate test vial was preserved with 3 drops of Lugol's iodine solution. The lids of the glass test vials were replaced and agitated to loosen the algae and ensure uniform preservation for later identification. After the preserved samples had settled, 10ml of liquid was poured from each test vial, and the settled benthic diatoms were carefully transferred into a 10mL sample storage tube.

Identification of Diatoms

Diatoms were identified by observation under an Olympus Bx50 Light microscope. Sub samples were taken from each replicate and observed in a Lund cell at a 400x magnification. Counting was conducted in random transects along the Lund cell until a total of at least 100 cells were counted and identified per replicate, which was sufficient for enumerating the common taxa in the sample, rare taxa that did not occur in every replicate were not included in analysis. Benthic diatoms were identified to the genus level using the following

international (Round et al. 1990, Cox 1996) and Australian keys (Gell 1999, Sonneman et al. 2000).

Health status of diatoms

The growth rates of the various diatoms differs substantially, and is very slow for some benthic taxa with doubling rates as low as $0.1-0.3 \text{ d}^{-1}$, this would make estimation of growth rate via cell counts difficult and lengthy (Admiraal 1976, Gould and Gallagher 1990). Therefore we have used a method of health classification similar to the live cell counts performed in other studies (Debenest et al. 2009, Pohlen et al. 2010, Proia et al. 2011), except data is recorded on a per taxa basis and includes identification of taxa as well as classification of health. The health status of the diatom cells was recorded as the number of diatom cells per genus that were either 'healthy' or 'unhealthy'. Cells were classified depending on the condition of the stained chloroplast. If it appeared more than 50% intact then it was classed as a healthy cell, and if the chloroplast was <50% intact or absent or the frustule was broken then it was classed as unhealthy (Appendix 1). Broken frustules were only counted if more than 50% of the valve was left intact and could be identified. Diatom community composition was calculated using only the healthy cells in order to determine the effects on the live benthic community. The percentage of healthy cells in each treatment was calculated as a proportion of the total number of cells counted in that treatment per genus.

Statistical Analysis

We assessed the effects of herbicide concentration on the health of diatoms using a generalised linear model (GLM). Concentration response of the diatom genera was performed using GLM on binary health data (healthy/unhealthy) using a logit link function. The model estimated the likelihood that a diatom cell would be healthy based on the concentration of exposure (50, 200, 500 $\mu\text{g/L}$) compared to the control parameter and was carried out on a per taxa basis. Where atrazine exposure resulted in a significant decline in diatom cell health the EC50 was calculated with nominal concentrations using Probit Analysis.

The health of the cells was also assessed at the start ($t=0$) and the end (48 hours) of the experiment to insure the stability of control health and to eliminate solvent effects. It was important to determine the background level of health for each genus, as this was expected to differ depending on the successional stage of the benthic diatom community at the time of collection (Davie et al. 2012). The background health of test controls (48 hour) was assessed using GLM as described above, compared to the start of experiment controls ($t=0$) as the reference parameter. Background health, concentration response and EC50 calculations were computed using SPSS 18 statistical package (SPSS 18).

Non-metric multi-dimensional scaling (MDS) ordination was conducted to examine community compositional changes of the healthy benthic diatom community among treatment groups at Bluewater Creek. MDS was conducted from the Bray Curtis index of similarity on untransformed community composition data. Only the community composition

data for the healthy cells was used in the MDS for the common taxa (taxa which were observed at least once in every sample). For the community response in the rapid test a one way ANOSIM was used to determine the differences in the healthy diatom community between treatments. SIMPER analysis was performed to determine which taxa contributed to the differences between groups. Multivariate statistical analysis was performed using PRIMER v6 (Clarke and Gorley 2006).

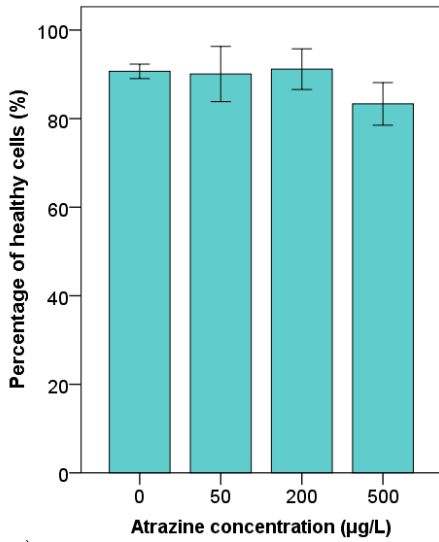
Results

Background health within control groups in rapid toxicity tests

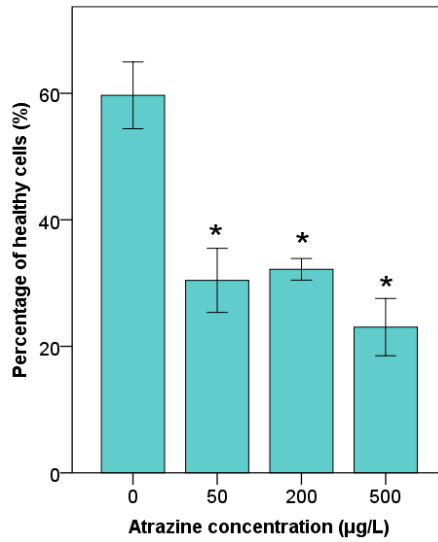
The background health of diatoms remained relatively consistent between controls across most genera from Bluewater Creek throughout the experiment. No solvent effect was observed for any of the taxa in the study. There were no differences between health of cells at the start of the experiment (t=0) and the controls (t=48) across all diatom genera (data not shown).

Concentration response and relative sensitivity of the diatom genera

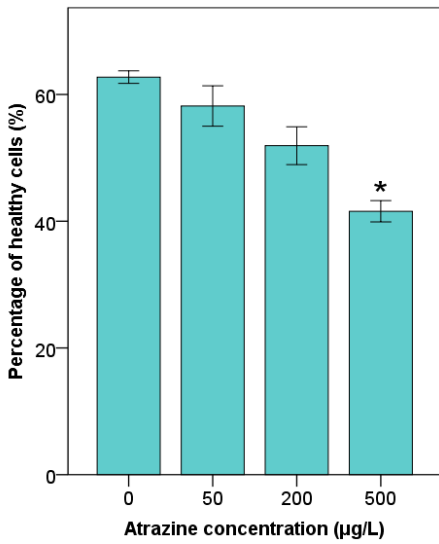
Differences in the relative atrazine sensitivity between benthic diatom genera were observed (Figure 1). The most tolerant genera were those which did not show a significant change in the number of healthy cells with herbicide exposure: *Navicula*, *Eunotia* and *Achnanthes* (Table 1). Diatoms from the genus *Navicula*, showed no concentration response to atrazine treatments and were the most tolerant in the benthic diatom community (Table 1 and Figure 1a). *Gomphonema* (Figure 1b) displayed a significant dose-response relationship to atrazine exposure and was the most sensitive taxa with an EC₅₀ of 43.5 µg/L and a significant concentration response at the lowest concentration treatment (Table 1). The most sensitive diatom genera within the benthic community were *Gomphonema*, *Ulnaria*, *Cymbella* and *Amphora*, all showed a significant concentration response effect at the highest concentration treatment of 500 µg/L (Table 1, Figures 1b, 1c, 1e and 1f). Compared to controls, the percentage of healthy cells declined significantly by 74% in *Gomphonema*, 62% in *Amphora*, 54% in *Cymbella* and 34% in *Ulnaria*, after exposure to the highest atrazine treatment (Table 1).



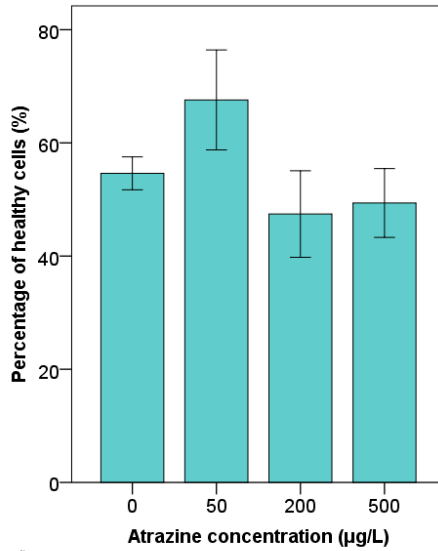
a)



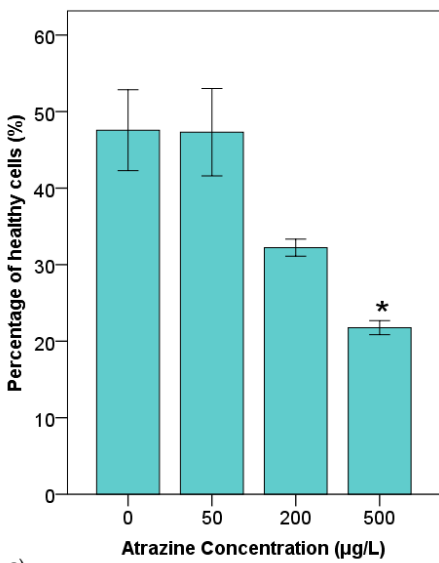
b)



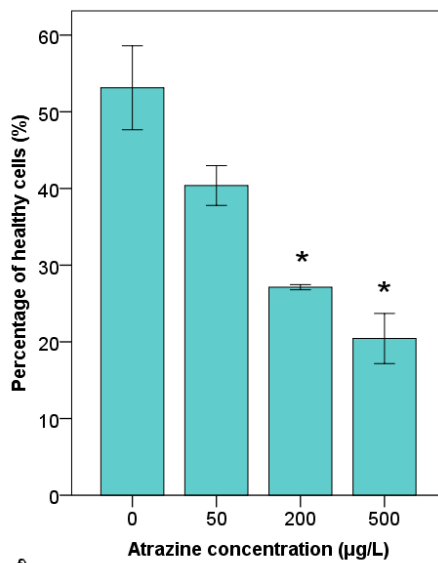
c)



d)



e)



f)

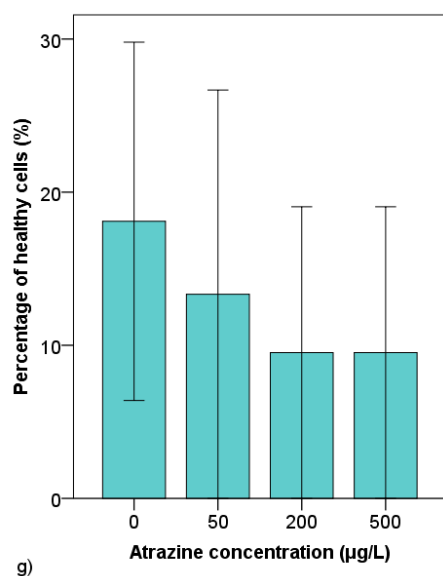


Figure 1 Graph of % of healthy diatom cells of a) *Navicula*, b) *Gomphonema*, c) *Ulnaria*, d) *Achnanthes*, e) *Cymbella*, f) *Amphora* and g) *Eunotia* within controls and atrazine treatments (50, 200, 500 µg/L) at 48 hours of exposure (Error bars represent ± 1 SE). Treatments marked * are statistically different from controls at alpha 0.05

Table 1 Concentration response of the diatom genera using the generalized linear model (GLM) effects of herbicide concentration on the health of diatom cells at each treatment level (50, 200, 500 µg/L atrazine) at 48 hours of exposure compared to controls (0 µg/L). Percentage of healthy cells per treatment, percentage composition of the healthy benthic diatom community, EC50 and EC10 values. - Not calculable.

Genus	Concentration (µg/L)	Sig.	Healthy cells (% $\pm$ SE)	Community composition (%)	EC50 (µg/L)	EC10 (µg/L)
<i>Navicula</i>	0	-	90.67 $\pm$ 1.62	13.12 $\pm$ 3.21	-	-
	50	0.808	90.08 $\pm$ 6.23	17.19 $\pm$ 0.91		
	200	0.905	91.17 $\pm$ 4.59	17.21 $\pm$ 0.86		
	500	0.403	83.33 $\pm$ 4.81	17.75 $\pm$ 2.06		
<i>Ulnaria</i>	0	-	62.74 $\pm$ 0.99	39.81 $\pm$ 1.57	1241	84.12
	50	0.434	58.18 $\pm$ 3.19	39.73 $\pm$ 1.31		
	200	0.059	51.92 $\pm$ 2.99	42.22 $\pm$ 2.82		
	500	0.000	41.56 $\pm$ 1.68	42.84 $\pm$ 1.33		
<i>Gomphonema</i>	0	-	55.03 $\pm$ 6.88	14.35 $\pm$ 2.03	43.47	0.123
	50	0.013	24.17 $\pm$ 6.14	10.44 $\pm$ 0.92		

Genus	Concentration (µg/L)	Sig.	Healthy cells (% ±SE)	Community composition (%)	EC50 (µg/L)	EC10 (µg/L)
<i>Achnanthyidium</i>	200	0.010	23.89 ±2.00	11.08 ±0.73		
	500	0.001	14.54 ±2.76	9.18 ±1.20		
	0	-	54.62 ±2.91	8.50 ±0.99	-	-
	50	0.437	67.59 ±8.83	10.18 ±1.08		
	200	0.429	47.43 ±7.64	11.79 ±1.80		
<i>Eunotia</i>	500	0.500	49.38 ±6.08	16.23 ±1.86		
	0	-	18.10 ±11.70	1.85 ±1.31	-	-
	50	0.367	13.33 ±13.33	0.97 ±0.97		
	200	0.485	9.52 ±9.52	1.13 ±1.13		
	500	0.485	9.52 ±9.52	1.19 ±1.19		
<i>Cymbella</i>	0	-	47.57 ±5.29	9.20 ±1.29	419.1	75.26
	50	0.995	47.31 ±5.70	7.51 ±0.59		
	200	0.193	32.22 ±1.11	6.78 ±0.52		
	500	0.027	21.77 ±.92	4.63 ±0.39		
	0	-	53.13 ±5.47	13.17 ±2.88	241.8	14.29
<i>Amphora</i>	50	0.119	40.38 ±2.59	13.98 ±3.10		
	200	0.002	27.12 ±.34	9.79 ±1.20		
	500	0.000	20.44 ±3.27	8.17 ±1.63		

Community effects of herbicide exposure

The Non-metric MDS ordination (stress = 0.09) showed a gradient of change in community composition of healthy benthic diatoms from the control groups to the highest herbicide exposure groups (Figure 2). Whilst some cross over between the controls and the lower concentration treatments was evident, the separation of the highest concentration treatment is evident and the ANOSIM showed significant differences between the treatment groups (Global R = 0.361, p-value = 0.005). There was a shift in the benthic community composition with increasing atrazine exposure, however the pairwise comparisons were not significant. The differences in community composition observed can be attributed to a decline in the most sensitive taxa and the increase of tolerant taxa after herbicide exposure (Table 1). The genera that had the greatest influence on the differences between the communities were *Amphora*, *Navicula* and *Ulnaria*, with each genus contributing approximately 19% to the Bray-Curtis dissimilarity between groups.

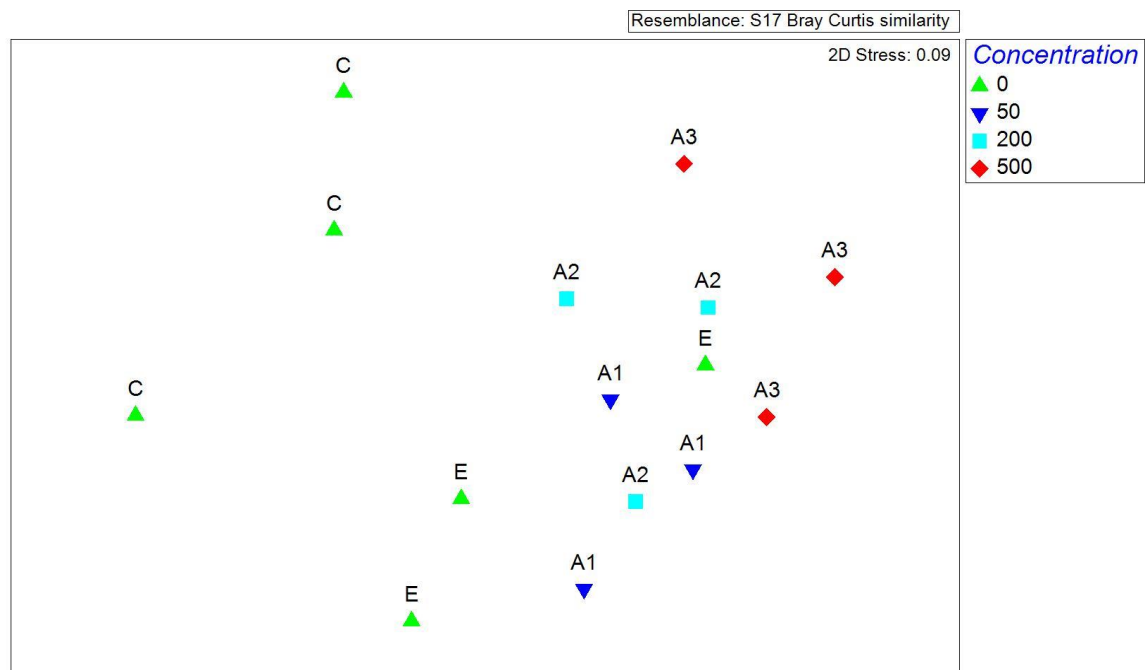


Figure 2 MDS ordination of healthy benthic community composition in the 48 hour atrazine exposure treatments, from Bluewater Creek diatom communities. Treatments: C = Control, E = Ethanol Control, A1 = 50µg/L atrazine treatment, A2 = 200µg/L atrazine treatment, A3 = 500µg/L atrazine treatment.

Discussion

A new method was established to determine the relative herbicide sensitivity of diatoms within a natural benthic community using rapid toxicity testing. The relative sensitivity of multiple diatom genera from a diverse field derived sample was determined from one 48 hour exposure test. This method is quicker and less costly than traditional methods of testing diatoms and algae which involve establishing cultures of each taxa and then testing each species individually (Peterson et al. 1997, Tang et al. 1997, Nelson et al. 1999, Magnusson et al. 2008, Magnusson et al. 2010, Brain et al. 2012a, Larras et al. 2012, Moro et al. 2012). The method used in this study is based on the rapid toxicity approach previously used with invertebrates (Kefford et al. 2003, Kefford et al. 2005a, Hickey et al. 2009). With the application of multiple rapid toxicity tests, herbicide sensitivity data for many taxa can be produced in a short period of time. This method is advantageous for the development of a traits based index, which would require sensitivity data for as many local taxa as possible.

Preliminary experiments were conducted in the development of this method that tested alternate methods for the collection of field derived natural benthic diatom communities for use in rapid toxicity tests. One alternate method used artificial glass slides as substratum for algae collection, requiring a colonisation period, multiple sites visits and specialised equipment (Guasch and Sabater 1998, Laviale et al. 2011, Proia et al. 2011). We encountered a number of logistical draw backs with the use of this substrate method in the study region of the tropics in North Queensland, Australia. A number of substrate cages containing glass

slides were lost or buried by substrate during the colonisation period, due to the extremity of flow events. Additionally, cages were damaged by vandalism. This resulted in only 3 of 9 cages deployed remaining intact and visibly patchy distribution of diatom growth on the remaining samplers. This approach was subsequently abandoned in favour of the scrubbing method of benthic diatom collection described in this study, which was quicker, requiring no prior site visits, and less expensive, requiring no specialised equipment.

A second alternate method using rock substrates collected *in situ*, was also tested. However, it was very difficult to obtain a high number of similarly sized and shaped rocks and to transport them to the laboratory without disturbance. We observed a very low density of diatoms on the small pebbles collected during the study, and since the purpose of retrieving pebbles from the field was to obtain a natural benthic community containing as many taxa as possible, this method was deemed unsuitable. Furthermore the diatom flora of small pebbles may only represent taxa that are rapid colonisers and may not reflect the general diatom community at a site due to the frequent movement and burial thereby resetting the colonisation process (Davie et al. 2012). Another unsuitable method was tested that used a 'rock cutter', a chisel and hammer, to break up large cobbles and boulders into smaller more manageable pieces. The benefits of this method would have been obtaining intact benthic diatom communities from larger rocks and thus at more advanced stages of colonisation. However, there were substantial practical difficulties of using the cutter and consequently it was not possible to obtain adequate sized rock shards with attached benthic diatom communities.

In order to overcome the difficulties with the above mentioned sampling methods, the current method was determined to be more suitable as it enabled benthic diatom communities to be collected without prior visits to a site or the use of *in-situ* samplers, or specialised equipment. In addition, it does not require the use of cultures to derive a representative community using multiple single species tests, which enhances the ecological relevance of the toxicity tests (Debenest et al. 2009). The results derived using this method showed limited variation of healthy cells in the controls over a 48 hour exposure, validating this method for comparisons between treatments and controls. This method enabled the relative sensitivities of multiple taxa in the natural benthic diatom community to be determined from one 48 hour rapid test.

This study identified differences in the herbicide sensitivity of freshwater diatom taxa within a natural benthic community. *Navicula* was the most tolerant genus to atrazine exposure and other genera such as *Ulnaria*, *Gomphonema*, *Cymbella* and *Amphora* were relatively more sensitive. These results are similar to Pesce et al. (2006) who in a mesocosm study found that *Navicula sp.* were more abundant than the other diatom taxa following exposure to Diuron compared to the controls. *Navicula sp.* are considered in the literature to be tolerant of both nutrient and herbicide pollution (Guasch and Sabater 1998, Chalifour and Juneau 2011). However, Magnusson et al. (2010) found that photosynthetic inhibition occurred in *Navicula sp.* at atrazine concentrations much lower than the exposures in this study. The concentrations of atrazine in the current study (50-500µg/L) exceed the field measured peak concentrations which regularly reach 10µg/L in rivers that flow into the Great Barrier Reef (GBR) (Brodie et al. 2012, Lewis et al. 2012, Smith et al. 2012). However, considering that photosystem II inhibitors (PSII) such as atrazine often occur in mixtures of two or more and that their toxicity is additive (Magnusson et al. 2010), recent studies have

calculated the atrazine equivalent concentrations of PSII herbicide mixtures to be up to 807µg/L at sites within the GBR catchment area (Smith et al. 2012), justifying the ecological relevance of these results in identifying which taxa are most at risk of herbicide toxicity in field derived communities.

Open questions include: (1) whether the diatoms cells which appeared healthy were physiologically impaired and (2) whether those individuals regarded as unhealthy would recover following the cessation of herbicide exposure. Prosser et al. (2013) observed rapid recovery of quantum yield from periphyton communities after the cessation of atrazine exposure with $\geq 95\%$ recovery within 48 hours and other studies have observed similar rapid recovery in quantum yield (Laviale et al. 2011, Brain et al. 2012a). However, as those studies did not investigate changes in cell health, it is uncertain what relevance they have for the recovery of diatoms classified as unhealthy in the current study. Indeed other studies (Dorigo et al. 2010, Magnusson et al. 2012) have observed much slower recovery of periphyton community structure following exposure to herbicides in the field (Morin et al. 2010). Such studies suggest that alternative approaches, for example the changes in cell health used in the current study, should also be investigated. Indeed, the ability of certain diatoms to recover after herbicide exposure may be an important trait for consideration alongside sensitivity in the development of a traits-based monitoring index using diatoms (Gustavson et al. 2003). The results of the relative sensitivity by diatom genera is meant as a means for ranking the relative sensitivities of the taxa or for classifying their sensitivity (e.g. sensitive or tolerant) and not as an indication of what atrazine concentration will or will not harm diatom taxa in nature.

Conclusions

The current study developed a new method of producing sensitivity data for a range of individual diatom taxa from within a natural benthic community in a short period of time. The rapid toxicity tests provided consistent control data with a low variability in the proportion of healthy cells per genera at the start of the experiment, which was suitable for determining the differences in the relative sensitivity of diatom genera to atrazine exposure. This method can deliver sensitivity data for multiple taxa from the one 48 hour test, without the need for cultures or multiple site visits, and will be useful for the production of herbicide sensitivity data that can be used for a new traits based index that can detect herbicide toxicity using benthic diatoms. We thus recommend the use of this method for conducting rapid toxicity testing of diatoms.

Chapter 2 – Is the relative sensitivity of benthic diatoms altered by herbicide type?

Abstract

Benthic diatoms are important phototrophic organisms and have the potential to be used as bioindicators of herbicide toxicity in the field. Herbicides are common pollutants of rivers associated with agricultural regions and occur as mixtures including differing modes of action. Whether herbicides of differing modes of action can alter the relative sensitivity of benthic diatoms within a natural benthic community is unknown. We used rapid toxicity testing to produce relative sensitivity data for multiple diatom taxa, within a natural benthic community, to eight common herbicides with differing modes of action; the photosystem II (PSII) inhibitors: atrazine, hexazinone, tebuthiuron, simazine and diuron; two auxin regulators: MCPA and 2,4-D; and an enzyme inhibitor: glyphosate. We found that the relative herbicide sensitivities of the taxa were not altered by the different herbicides. The most sensitive taxa were *Amphora* and *Gomphonema cf. angustatum*, whereas *Navicula* was unresponsive to most herbicides even at the highest concentration tested, 500 µg L⁻¹. These results reveal a trend of relative sensitivity between benthic diatoms that is separate from effects of herbicides' modes of action. This indicates freshwater benthic diatoms may be suitable as *in situ* indicators for detecting toxicity of multiple herbicides, including those with differing modes of action.

Introduction

Freshwater benthic diatoms are important phototrophic organisms of lotic and lentic environments and have the potential to be used as *in situ* indicator communities of herbicide pollution. Agricultural herbicides are common pollutants of freshwater environments (Lewis et al. 2009, Davis et al. 2011) and can alter the growth and physiology of freshwater benthic diatoms, as well as the structure and diversity of benthic communities (Morin et al. 2009, Debenest et al. 2010, Magnusson et al. 2012). Benthic diatoms are widely recognised as effective biomonitors as they respond rapidly to changes in water quality. However, a monitoring tool using diatoms as indicators of herbicide toxicity has not yet been established. Assessing the ecological effects of herbicide contamination is essential in protecting freshwater ecosystems from degradation and changes in the benthic diatom community may enable the early detection of toxic impacts (DeLorenzo et al. 2001, Ricart et al. 2009). The herbicide sensitivity of diatoms differs greatly between taxa (Debenest et al. 2009), and this trait has the potential to be used to link shifts in community composition with herbicide contamination in the field (Schäfer et al. 2011b). However, sensitivity traits data for freshwater benthic diatoms to herbicides is limited and this is a barrier to the development of a biomonitoring tool capable of diagnosing herbicide toxicity in the field (Roubeix et al. 2011).

Agricultural herbicides are often detected in waterways as mixtures and rarely occur alone (Magnusson et al. 2010). The various types of herbicides can be classified by their modes of action, the mechanism by which they act on a target organism. Photosystem II (PS-II) inhibitors, which include atrazine, simazine, hexazinone, tebuthiuron and diuron, are a commonly detected type of herbicide in agricultural regions of the Great Barrier Reef (GBR) catchment area. These herbicides act on the photosystem II reaction centre by blocking electron transport and halting photosynthesis, ultimately resulting in oxidative stress and cell death (Rutherford and Krieger-Liszka 2001). Other common herbicidal modes of action include the enzyme inhibitors, such as glyphosate which cause chlorophyll degradation and reduced photosynthesis (Malik et al. 1989, Baylis 2000) and the auxin growth regulators such as MCPA and 2,4-D.

Very few studies have investigated whether exposure to different herbicides alters the relative sensitivity of diatoms, or whether the differences in sensitivity between taxa are the same across different herbicides with the same or differing modes of action. It is therefore important to obtain relative sensitivity data for a wide range of herbicides for as many local taxa as possible, and to assess whether or not the relative sensitivity differs with herbicides of different modes of action. This will determine the suitability of freshwater benthic diatoms as an indicator capable of detecting toxicity from multiple herbicides including across differing modes of action.

The aim of this study was to determine whether the relative herbicide sensitivity of benthic diatom taxa differs between herbicides with the same or different mode of action. We compared relative sensitivity data for multiple diatom taxa collected *in situ* from a natural benthic community to eight common herbicides. The herbicides included in this study are the PS-II inhibitors atrazine, hexazinone, tebuthiuron, simazine and diuron, two auxin regulators MCPA, 2,4-D, and an enzyme inhibitor glyphosate. This study used rapid toxicity tests to expose freshwater benthic diatom communities to the herbicides. The relative

sensitivities of the diatom taxa within the natural benthic community will be compared for each herbicide to assess whether tolerant taxa are the same across all eight herbicides or whether the relative sensitivities of the taxa are dependent on herbicide mode of action.

Materials and methods

The following experiment was conducted at the same time and date as part of the previous chapter and replicates some of the data and analysis. All experimental procedures are identical to the previous chapter, noting the following exceptions.

Rapid toxicity tests

The benthic diatoms were exposed over 48 hours to eight herbicides; atrazine, simazine, hexazinone, tebuthiuron, glyphosate, diuron, MCPA and 2,4-D.

The solution containing the removed benthic diatom community from Bluewater Creek was homogenised by gentle shaking and divided into 1mL aliquots randomly assigned to 81 x 40mL test vials by pipette.

All herbicide stock solutions were prepared in site water using a carrier of 99% ethanol to increase the solubility (2% v:v) except Glyphosate which did not require a carrier.

There were 3 herbicide exposure treatments, consisting of herbicide concentrations of 50, 200 and 500 µg/L, which were shown to elicit a response in the sensitive taxa from trial tests (data not shown).

Statistical Analysis

We assessed the effects of herbicide concentration on the health of diatoms using a generalised linear model (GLM). Concentration response of the diatom genera was performed using GLM on binary health data (healthy/unhealthy) using a logit link function. The model estimated the likelihood that a diatom cell would be healthy based on the concentration of exposure (50, 200, 500 µg/L) compared to the control parameter and was carried out on a per taxa basis. Where atrazine exposure resulted in a significant decline in diatom cell health the EC50 was calculated with nominal concentrations using Probit Analysis.

The health of the cells was also assessed at the start ($t=0$) and the end (48 hours) of the experiment to insure the stability of control health and to eliminate solvent effects. It was important to determine the background level of health for each genus, as this was expected to differ depending on the successional stage of the benthic diatom community at the time of collection (Davie et al. 2012). The background health of test controls (48 hour) was assessed using GLM as described above, compared to the start of experiment controls ($t=0$) as the reference parameter. Background health, concentration response and EC50 calculations were computed using SPSS 18 statistical package (SPSS 18).

Results

Background health within control groups in rapid toxicity tests

There was little difference in the health of cells between the three control treatments with no difference between the level of health at the start of experiment ($t=0$) and the control at 48 hours, in any taxa (data not shown). Most taxa had levels of control health above 47% except for *Eunotia* which had very low background health levels (18.1%). The proportion of healthy *Achnanthes* cells appeared to increase over the course of the experiment, although this not significant.

Herbicide type on relative sensitivity

Relative sensitivity of the benthic diatom taxa was not dependant on herbicide mode of action. For the majority of taxa, the herbicide type was not important in predicting effects on diatom health, whereas exposure concentration was predictive of diatom health (Table 2). The GLM found that concentration was a significant predictor variable of health in the majority (7 of 8) of taxa, whereas herbicide type only had an effect in two taxa; *Ulnaria* and *Gomphonema cf. angustatum*. There was no significant interaction between concentration and herbicide type for any taxa. The relative sensitivity of the taxa differed very little between herbicides. The ranking of the taxa by EC50 (Figure 3) shows the most sensitive taxa *Amphora* and *Gomphonema cf. angustatum* are the same regardless of herbicide type. *Navicula* was by far the most tolerant taxa in 6 out of 8 herbicides and was ranked among the most tolerant across all herbicides (Figure 1).

Table 2 GLM results for effects of herbicide type and concentration on the health of diatom cells, and the interaction between herbicide type and concentration response.

Taxa	Treatment	Wald Chi-Square	df	Sig.
<i>Navicula</i>				
	Herbicide	5.669	7	0.579
	Concentration	8.914	2	0.012
	Interaction	12.825	14	0.540
<i>Ulnaria</i>				
	Herbicide	21.433	7	0.003
	Concentration	30.157	2	0.000
	Interaction	9.828	14	0.775
<i>Gomphonema cf. lanceolatum</i>				
	Herbicide	10.812	7	0.147

Taxa	Treatment	Wald Chi-Square	df	Sig.
<i>Gomphonema cf. angustatum</i>	Concentration	2.804	2	0.246
	Interaction	10.262	14	0.743
	Herbicide	14.562	7	0.042
	Concentration	6.861	2	0.032
	Interaction	6.849	14	0.940
<i>Achnantheidium</i>	Herbicide	7.071	7	0.422
	Concentration	13.248	2	0.001
	Interaction	18.307	14	0.193
<i>Cymbella</i>	Herbicide	9.361	7	0.228
	Concentration	11.821	2	0.003
	Interaction	5.643	14	0.975
<i>Amphora</i>	Herbicide	7.405	7	0.388
	Concentration	37.822	2	0.000
	Interaction	4.721	14	0.989
Eunotia	Herbicide	5.675	7	0.578
	Concentration	0.000	2	1.000
	Interaction	3.628	13	0.955

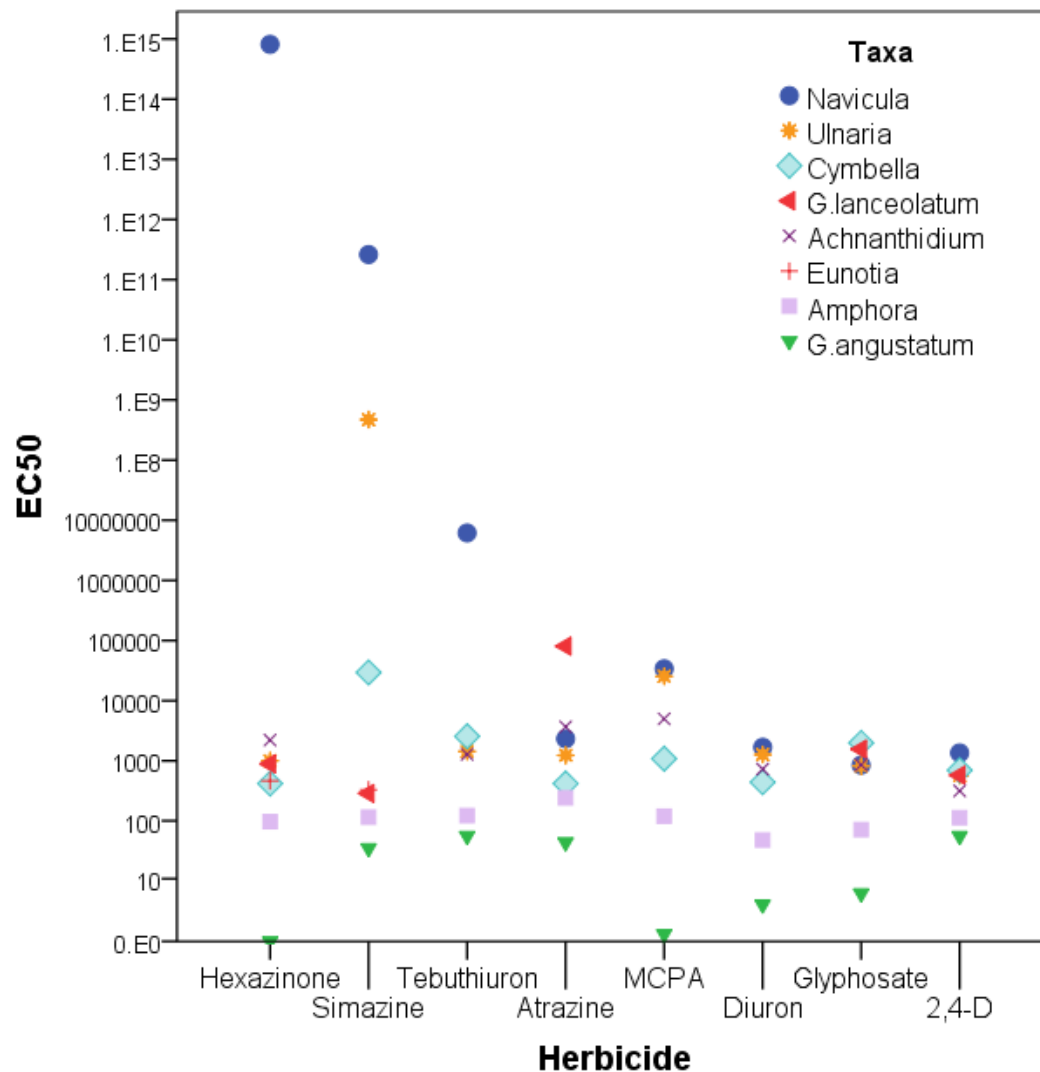


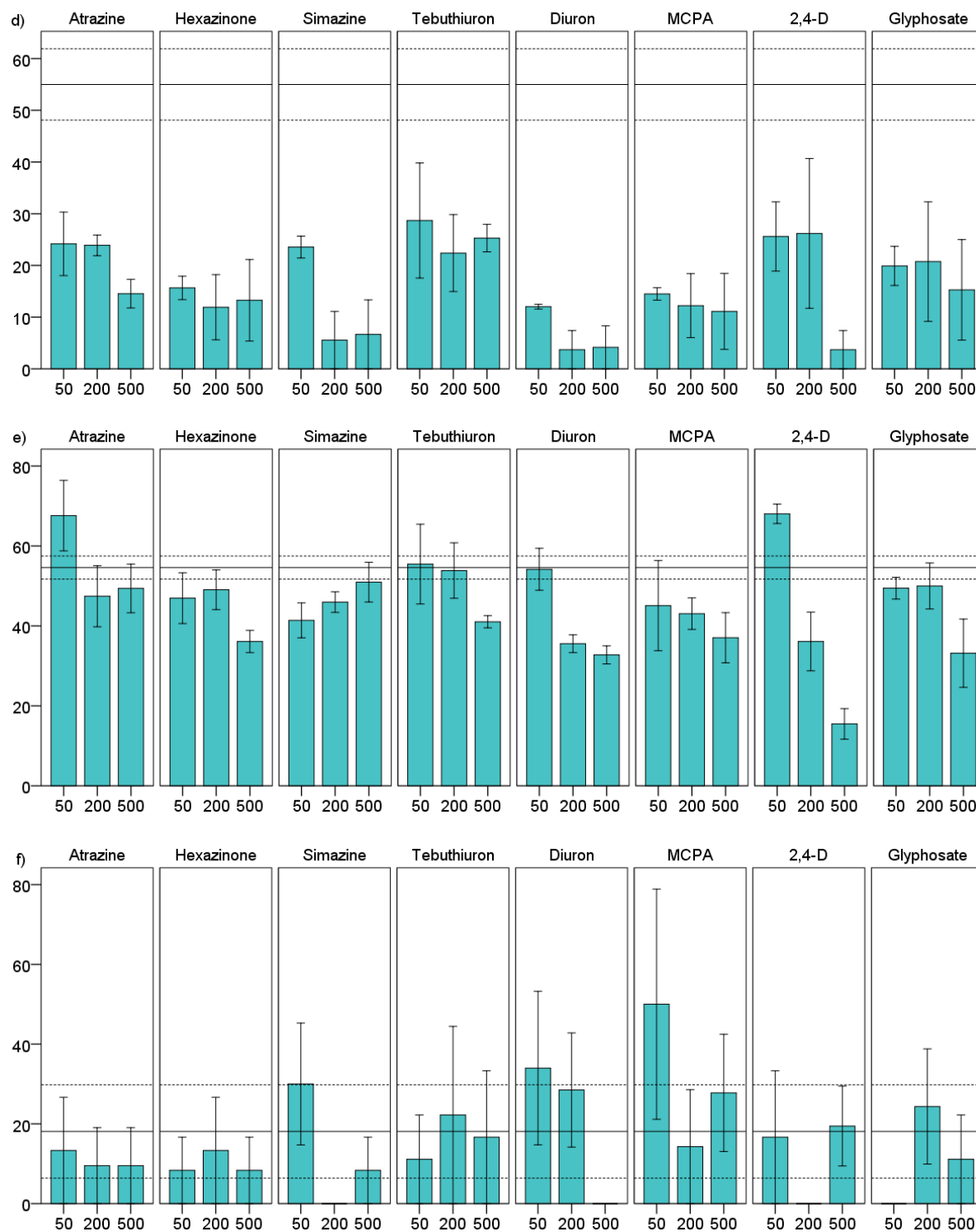
Figure 3 Ranking of the diatom taxa by EC50 for eight herbicides; atrazine, hexazinone, tebuthiuron, simazine, diuron, MCPA, 2,4-D, and glyphosate.

Relative herbicide sensitivity of the diatoms

Herbicide sensitivity differed substantially between the diatom taxa. *Navicula* was the least responsive taxa across all of the herbicides tested (Figure 4a). The health of *Navicula* cells was not affected by any of the herbicides at the lowest concentration treatment (50 µg/L) and only showed a slight concentration response at the highest concentrations of diuron (200 µg/L) and glyphosate (500 µg/L). However, even after exposure to high concentrations of diuron the proportion of healthy *Navicula* cells remained high (70.9% ± 0.5% SE). *Ulnaria*, *Gomphonema cf. angustatum* and *Amphora* were the most sensitive taxa showing a significant concentration response with increasing concentration treatments across all herbicides (Figures 4b, 4d & 4h). *Gomphonema cf. lanceolatum* was only sensitive to the PSII inhibitors at the highest concentrations, but was not sensitive to the growth regulators or the enzyme inhibitor, glyphosate (Figure 4c). *Cymbella* displayed a dose response to most herbicides; however this was only significant with the PSII herbicides atrazine, hexazinone

and diuron at the highest concentration treatments (Figure 4g). The response of *Achnanthyidum* to herbicide exposure was highly variable between treatments and herbicides (Figure 4e). There was an apparent concentration response in high herbicide treatments across all herbicides except simazine, but the effect was only significant for diuron. There was no concentration effect at any treatment level in *Eunotia* cells, however the SE in some treatments was greater than the mean proportion of heath (Figure 4f), which was very low even in controls so it is difficult to discern any difference between treatments.





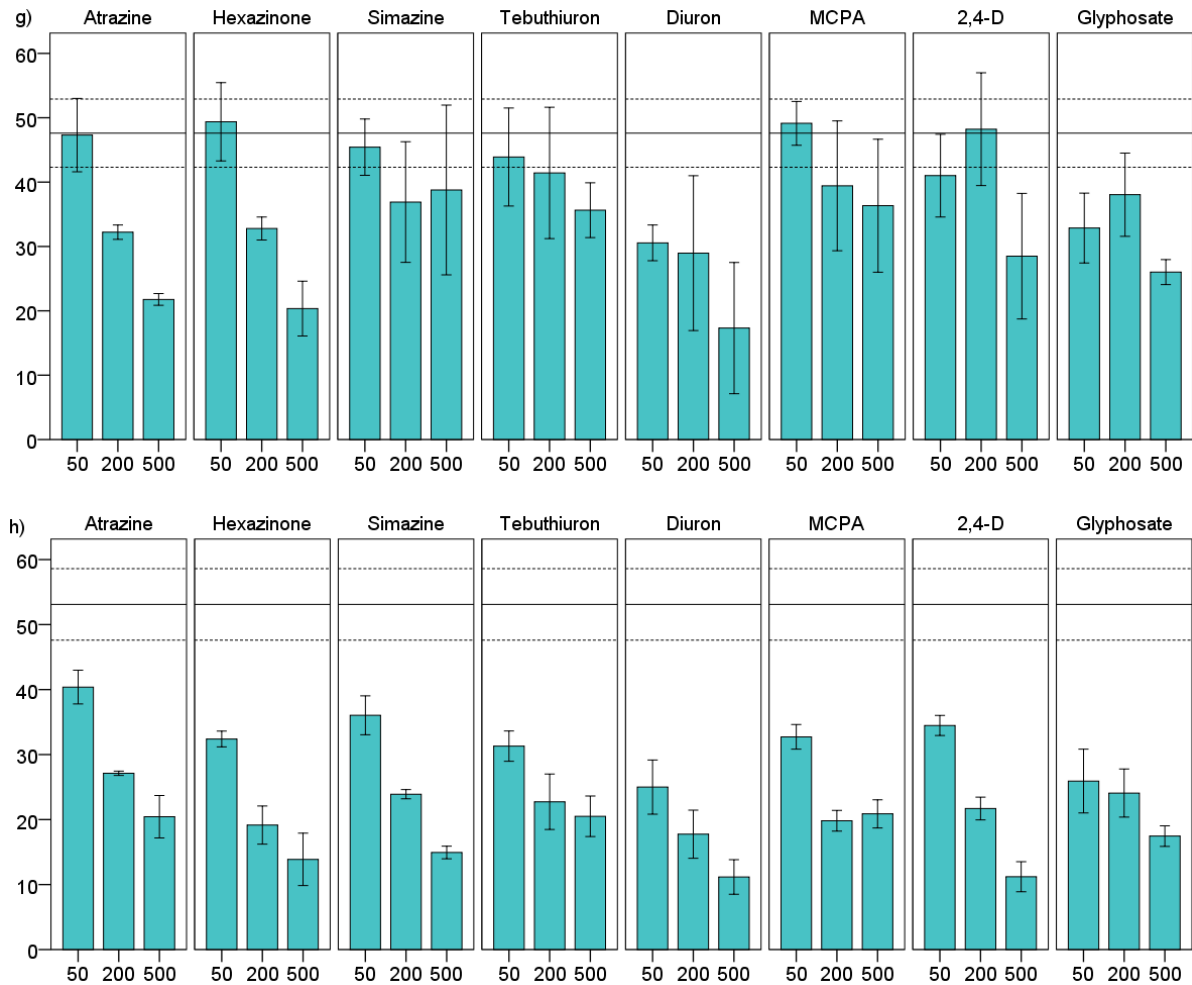


Figure 4 Concentration effects on the health (%) of cells per of herbicide treatment (50, 200, 500 µg/L) between the different herbicides at 48 hours of exposure for each diatom taxa a) *Navicula* b) *Ulnaria* c) *Gomphonema cf. lanceolatum* d) *Gomphonema cf. angustatum* e) *Achnanthisdium* f) *Eunotia* g) *Cymbella* h) *Amphora*. Mean response of the ethanol control treatment is shown as a solid horizontal line with 95% confidence intervals shown as dashed lines.

Discussion

Effects of herbicide type on the relative sensitivity of the diatoms

We exposed diatoms within a natural benthic community to eight different herbicides in rapid toxicity tests and the relative sensitivity of the diatoms was not altered by herbicide mode of action. For the majority of taxa, herbicide concentration was the only factor predictive of diatom health. The taxa *Ulnaria* and *Gomphonema cf. angustatum* were the only taxa to show effects of herbicide type on cell health and were some of the more sensitive taxa in the study. However, *Gomphonema cf. angustatum* was the most sensitive taxa to all herbicides and its sensitivity relative to the other taxa was unchanged by herbicide

type (Figure 3). It may be that herbicide effects were only apparent in the most responsive taxa, and that regardless of herbicide type the tolerant taxa were unresponsive. Whilst this study only represents some of the dominant taxa present in this particular region, these results establish a trend of relative sensitivity that operates widely across herbicide modes of action. Very few other studies have investigated variations in relative sensitivity in freshwater benthic diatoms between different herbicides. Despite this, there are some authors which have shown similar patterns to these. Larras et al. (2012) found that the motile species (*Craticula accomoda*, *Eolimna minima*, *Mayamaea fossalis* and *Nitzschia palea*) were the most tolerant regardless of herbicide type, and that species sensitivity distributions (SSD) were similar between different PSII inhibitors. Debenest et al., (2009) found that benthic diatom communities followed the same community structure after exposure to both a growth inhibitor s-metolachlor and the PSII inhibitor isoproturon.

Relative sensitivity of the diatoms

Within the natural freshwater benthic community there may be various diatom taxa present representing a wide range of physiological sensitivities to herbicides. Indeed, in this study, the herbicide sensitivities between taxa varied. We favoured the use of benthic diatom communities collected *in-situ* to improve environmental relevance and to represent the response of an actual riverine community (Debenest et al. 2009). It is expected that these results will contribute towards the classification of sensitivity traits data for a broad range of diatom taxa, which can be used to identify taxa most at risk of herbicide toxicity in rivers. In this study the sensitive taxa were *Amphora*, *Gomphonema cf. angustatum*, *Gomphonema cf. lanceolatum*, *Cymbella* and *Ulnaria*. The most tolerant taxa across all herbicides were *Navicula* and *Eunotia*. *Achnantheidium* was difficult to classify due to variability between treatments (Figure 4) and the increase in the proportion of healthy cells within control treatments (data not shown). It appears that *Achnantheidium* is more sensitive to herbicide exposure compared with the most tolerant taxa in the study and more tolerant than the most sensitive taxa (Figure 3). The two *Gomphonema* taxa showed different sensitivities; *Gomphonema cf. angustatum* was a more sensitive species than *Gomphonema cf. lanceolatum*. This highlights the possibility that depending on the dominant species within the benthic community, the relative ranking of the diatom by genera could differ. However, in the context of classification of the diatom genera as either sensitive or tolerant, the differences are minor. The most tolerant *Gomphonema* species in this study was still more sensitive relative to the tolerant taxa, such as *Navicula*, across most herbicides (Figure 3).

A pattern of relative sensitivity in benthic diatoms is often related to the heterotrophic capabilities of particular diatom taxa. Some studies have highlighted the relationship between trophic mode of diatoms and tolerance to PS-II herbicides (Pérès et al. 1996, Debenest et al. 2009). The capability of some species of diatoms to switch trophic mode, from autotroph to heterotroph, allows them to cope with reduced photosynthetic efficiency and may thereby make them more tolerant to PS-II inhibitors. Facultative heterotrophs have been shown to be more tolerant to the photosynthesis inhibitor isoproturon compared to autotrophs (Pérès et al. 1996, Debenest et al. 2009). Heterotrophy is common in many *Naviculales*, and they are one of the most herbicide tolerant taxa in this and other studies (Guasch et al. 1998, Ricart et al. 2009, Larras et al. 2012). However the ability to infer

herbicide tolerance of the diatom taxa by using information on their heterotrophic abilities as a proxy needs to be further tested in field studies.

Conclusions

The relative sensitivity of the diatom taxa was not altered by herbicide mode of action. The taxa most sensitive to herbicide toxicity were *Gomphonema cf. angustatum*, *Amphora*, *Cymbella* and *Ulnaria* and this was consistent between the eight herbicides tested. The consistency of the sensitivity data between the herbicides tested is significant for the use of diatoms as potential indicators of toxicity to broad range of herbicides in the field.

Chapter 3 - Does light intensity affect relative herbicide sensitivity of freshwater benthic diatoms?

Abstract

Benthic diatoms are ubiquitous in streams associated with agricultural lands and are at risk of phytotoxic effects of herbicides. Biomonitoring of community compositional changes in benthic diatoms could be used to predict herbicide effects in the field. We are measuring relative herbicide sensitivity in a large number of local species to develop a biomonitoring index which compares the abundance of sensitive and tolerant diatoms in a community. Light availability is a determinant of primary productivity in benthic communities and may alter the relative herbicide sensitivities of the diatom taxa within the benthic community. That is a species which are herbicide tolerant (relative to other species) under high light may be relatively sensitive under low light. This study compares the relative sensitivities of taxa within a multispecies benthic diatom community under different light exposure scenarios. Benthic diatoms from natural communities were collected *in situ* and exposed to the herbicides Atrazine and Glyphosate, under high light ($100 \mu\text{mol s}^{-1} \text{m}^{-2}$) and low light ($20 \mu\text{mol s}^{-1} \text{m}^{-2}$) conditions, using 48 hour rapid toxicity tests. The interaction of light and herbicide concentration was assessed for 32 benthic diatom taxa. Many of the diatom taxa appeared to be affected by lower concentrations of Atrazine and/or Glyphosate under low light. With statistically significant interactions between exposure concentration and light level in four taxa, *Ulnaria sp.*, *Gomphonema cf. olivaceum*, *Eunotia sp.*, and *Navicula cf. veneta*. However, in no cases did sensitive species appear to be tolerant, or *vice versa*, under altered light conditions. Therefore, it is unlikely that the overall ranking of relative sensitivity between taxa would be altered by light. The sensitivity data produced supports the suitability of benthic diatoms as indicators of herbicide toxicity in rivers.

Introduction

Light intensity may have an impact on the herbicide sensitivity of freshwater benthic diatoms. The availability of light is a major determinant of primary production and benthic community composition (Lange et al. 2011). The excess or limitation of light can be seen as a stressor that may influence the subsequent ability of benthic communities to tolerate additional stress events such as herbicide exposure (Bonnineau et al. 2012). Moreover, many common agricultural herbicides act on a target organism by halting the reactions that take place during photosynthesis. For example the triazine herbicides or photosystem II (PSII) inhibitors, which bind to the D1 protein, blocking electron transfer and preventing a redox reaction called the Hill reaction (Rutherford and Krieger-Liszkay 2001, Debenest et al. 2010). Since the mode of action is light dependent in PSII inhibiting herbicides, light conditions during exposure may alter herbicide sensitivity (Brain et al. 2012b).

The freshwater benthic community is one of the first to be exposed to herbicide pollution in rivers receiving agricultural run-off and may be early indicators of herbicide toxicity (Kosinski 1984). In order to develop new biomonitoring tools that can detect the impacts of herbicides in rivers using changes in benthic diatom communities, it is necessary to identify the species most at risk of herbicide impacts (Larras et al. 2014). As light intensity may affect herbicide sensitivity, it is important to determine if these factors interact for as many freshwater benthic diatom taxa as possible. Altered sensitivity to herbicides under differing light intensities has been demonstrated in the green algae *Chlamydomonas reinhardtii* (Fischer et al. 2010) and *Nannochloris oculata*, the freshwater aquatic macrophyte *Elodea canadensis* (Brain et al. 2012b), the planktonic diatoms *Fragilaria crotonensis* and *Aulacoseira granulata* var. *angustissima* (Deblois et al. 2013) and also in the marine diatom *Phaeodactylum tricornutum* (Mayasich et al. 1986). However, studies investigating interactions between light intensity and herbicide sensitivity in freshwater benthic diatoms is nonexistent. Additionally, the effects that light condition during herbicide exposure may have on benthic communities in the field are poorly understood.

Therefore, this study compares the effects of altered light on the relative herbicide sensitivity of freshwater benthic diatoms, for two herbicides with differing modes of action, glyphosate, an enzyme inhibitor and the PSII inhibitor, atrazine. Diatoms from four freshwater benthic communities collected *in situ* were exposed to herbicides in 48 hour rapid toxicity tests under different experimental lighting conditions; high light (100 $\mu\text{mol}/\text{m}^2/\text{s}$) and low light (20 $\mu\text{mol}/\text{m}^2/\text{s}$). The effects of light and herbicide concentration on cell health were assessed in 28 benthic diatom taxa. Whilst it is expected that light conditions will alter the absolute sensitivities of individual diatom taxa, it is not known whether this will affect the relative sensitivities of species within the benthic community. The purpose of this study was to determine; 1) if there was an interaction between light intensity and herbicide concentration effects in as many freshwater benthic diatom taxa as possible and 2) whether the relative herbicide sensitivity of the diatom taxa within a natural benthic community was altered by light conditions during exposure. The sensitivity data produced will be used to determine which species are most at risk of herbicide toxicity within the benthic diatom community.

Material and Methods

Diatom collection locations

Natural benthic diatom communities were collected from four rivers that drain into the Great Barrier Reef World Heritage Area (GBRWHA). Collection occurred between 24th October 2012 and 5th November 2012.

Alligator Creek is situated 30 km southeast of Townsville in the dry tropics climatic region of north Queensland (19°25.777'S, 146°56.599'E). It is part of the Ross River subcatchment with its source in Bowling Green Bay National Park. The creek is approximately 10 meters wide with predominantly large cobbles, boulders and bedrock substrate. The surrounding vegetation is dense eucalypt woodland and pockets of rainforest upstream. The site was chosen to represent a minimal impact site with only recreational activities occurring upstream of the site and negligible agricultural impacts such as herbicide pollution.

Barratta Creek is located 70 km southeast of Townsville in the dry tropics (19°42.416'S, 147°08.850'E). The site is located in the subcatchment area for the Lower Burdekin Basin, land uses in the basin are predominantly agricultural including grazing and cropping. The river has elevated concentrations of herbicide contaminants (Davis et al. 2008). The site therefore represents a highly impacted river with past herbicide contamination events. Collection occurred upstream of a road bridge and rail bridge river crossing. The river is approximately 7 meters wide with a narrow vegetation corridor either side of the river consisting of eucalypts and grasses. The substrate consists of cobbles, pebbles, gravel and sand. Some areas of the river edge are susceptible to erosion.

Gowrie Creek is located 45 km west of Ingham in the wet tropics (18°26.856'S, 145°50.873'E). Gowrie creek is part of the Herbert River Basin, its headwaters originate in Girringun National Park before joining the Herbert River downstream of the collection site. Land use in the catchment area is dominated by grazing, and secondarily by cropping. The river is 10 meters wide and sparsely vegetated with grasses, shrubs and small trees. The substrate consists of mainly small cobbles, pebbles and gravel.

Liverpool Creek is 30 km south west of Innisfail in the wet tropics bioregion (17°43.432'S, 145°55.999'E). Part of the South Johnston River subcatchment area its headwaters originate in Japoon National Park, the surrounding land use is mostly cropping and grazing. The collection site was located at a rest stop adjacent to the river's edge. The river's edges are sparsely vegetated with trees, reeds, grasses and shrubs and the substrate is cobbles and pebbles with a channel width of 20m.

Sampling of natural benthic communities

The same method was used as in the previous chapter.

Rapid toxicity tests

The experimental procedure was the same as the previously described chapter aside from the following exceptions. Two rapid toxicity tests were conducted in a control temperature laboratory at 24 ± 2 degrees Celsius. Both tests used two experimental light irradiance levels on a 12 hour light:dark cycle; low light intensity of $20 \mu\text{mol s}^{-1} \text{m}^{-2}$ ($\pm 10\%$) or high light intensity $100 \mu\text{mol s}^{-1} \text{m}^{-2}$ ($\pm 10\%$). The lighting conditions were achieved with lamps (Pierlite 36W/ 840 4000k) suspended at various heights to reach the required irradiance. Irradiance was measured using a PAR meter (LI-190 Quantum Sensor) held in the same position where the rapid tests occurred. Experimental test vials containing the algae were made up to a final volume of 10mL with site water spiked with a known herbicide concentration depending on treatment. All control and herbicide treatments were duplicated under each light level, high and low light.

Two rapid toxicity tests were conducted. The first test used benthic diatom communities collected from Gowrie Creek and Liverpool Creek, which were exposed to atrazine at concentrations of 20, 50, 200 and 500 $\mu\text{g/L}$. In the second test diatom communities from Alligator Creek and Barratta Creek were exposed to either atrazine or glyphosate at concentrations of 50, 200 and 500 $\mu\text{g/L}$. A carrier of ethanol (99%) was used for atrazine, whereas glyphosate did not require a carrier. Atrazine treatments were compared to ethanol controls (v/v % 0.05 ethanol, no herbicide), and glyphosate treatments were compared to site water only controls (no ethanol, no herbicide) both treatments are hereafter referred to as 'controls'.

Preservation, identification and health status of diatoms

At the end of the test period the diatoms were preserved, identified and classified as described in the previous chapter. Identification and health classification of the taxa in this study are described in Appendix 1.

Statistical analysis

We assessed the effects of herbicide concentration on the health of diatoms using a generalised linear model (GLM). Concentration response of the diatom genera was performed using GLM on binary health data (healthy/unhealthy) using a logit link function. The model assessed effects of both concentration of exposure and light level during exposure on cell health and also the interaction of the two factors, compared to the control parameter and was carried out on a per taxa basis.

The health of the cells was also assessed at the start ($t=0$) and the end (48 hours) of the experiment to insure the stability of control health and to eliminate solvent effects. The background health of test controls (48 hour) was assessed using GLM, compared to the start of experiment controls ($t=0$) as the reference parameter. Graphical and statistical analysis was computed using SPSS 18 statistical package (SPSS 18).

Results

Background health within control groups

Levels of healthy cells between control treatments were uniform throughout the experiment in most taxa (data not shown). The control health of *Gomphonema cf. olivaceum*, *Gomphonema cf. gracile*, *Ulnaria sp.*, *Eunotia sp.* and *Eunotia spp.* varied over the course of the experiment. Some taxa had low proportion of healthy cells (<30% healthy) at the start of the experiment; *Cymbella sp.*, *Cymbella cf. lanceolata*, *Encyonema sp.* *Epithemia* and *Ulnaria ulna*.

Influence of light on diatom sensitivity

There was no significant interaction between herbicide concentration response and light condition during exposure for the majority of taxa (Table 3). The taxa in which herbicide sensitivity was dependent on light level were *Ulnaria sp.*, *Gomphonema cf. olivaceum*, *Eunotia sp.*, and *Navicula cf. veneta* (4 out of 32 taxa). For two of these taxa, *Ulnaria sp.* and *Gomphonema cf. olivaceum*, the interaction of effects were seen in atrazine but not in glyphosate (Figure 5), only atrazine was tested on the other two taxa. Declining cell health after atrazine exposure was more likely under low light conditions in *Gomphonema cf. olivaceum* (Figure 5a), *Eunotia sp.*, and *Navicula cf. veneta*. Conversely, a decline in the health of *Ulnaria sp.* with increasing atrazine concentration exposure corresponded with high light conditions (Figure 5b). A number of other taxa appeared to be more sensitive to atrazine under low light conditions, *Cocconeis cf. placentula*, *Ulnaria ulna*, *Encyonema sp.*, *Ulnaria ulna*, *Nitzschia spp.*, *Cymbella sp.*, *Gomphonema cf. parvulum*, *Epithemia*, *Eunotia*, *Gomphonema cf. gracile*, *Ulnaria cf. acus*, however the interaction was not significant.

Table 3 Results of the GLM analysis on the effect of light and herbicide concentration on the health of diatom cells, and the interaction of light and concentration response. – Not tested.

Taxa	Site	Atrazine			Glyphosate		
		Concentration	Light Level	Interaction	Concentration	Light Level	Interaction
1. <i>Cocconeis sp.</i>	Barratta Ck	0.920	0.272	0.860	1.000	1.000	1.000
2. <i>Cymbella cf. lanceolata</i>	Barratta Ck	1.000	1.000	1.000	1.000	1.000	1.000
3. <i>Gomphonema cf. parvulum</i>	Barratta Ck	0.491	0.268	-	0.984	-	-
4. <i>Gyrosigma sp.</i>	Barratta Ck	1.000	1.000	1.000	1.000	1.000	1.000
5. <i>Melosira cf. varians</i>	Barratta Ck	0.007	0.064	0.644	0.498	0.999	0.850

Taxa	Site	Atrazine			Glyphosate		
		Concentration	Light Level	Interaction	Concentration	Light Level	Interaction
							0
6. <i>Melosira</i> sp.	Barratta Ck	0.010	0.220	0.751	0.041	0.924	0.088
7. <i>Naviculacf. shroerti</i>	Barratta Ck	0.810	0.726	0.754	0.608	0.853	0.895
8. <i>Navicula</i> cf. <i>rostellata</i>	Barratta Ck	0.517	0.800	0.576	0.931	0.293	0.732
9. <i>Navicula</i> cf. <i>veneta</i>	Barratta Ck	0.984	0.999	0.913	0.979	0.999	0.784
10. <i>Pinnularia</i> sp.	Barratta Ck	1.000	1.000	1.000	1.000	1.000	1.000
11. <i>Ulnaria</i> sp.	Barratta Ck	<0.0001	0.081	<0.0001	0.001	0.072	0.207
12. <i>Ulnaria ulna</i>	Barratta Ck	0.037	0.577	0.750	0.725	0.820	0.566
13. <i>Cymbella</i> cf. <i>lanceolata</i>	Alligator Ck	0.014	0.474	0.620	0.024	0.999	0.998
14. <i>Epithemia</i> sp.	Alligator Ck	0.157	0.358	0.809	0.253	0.646	0.818
15. <i>Eunotia</i> cf. <i>minor</i>	Alligator Ck	0.080	0.854	0.408	0.255	0.372	0.438
16. <i>Gomphonema</i> cf. <i>gracile</i>	Alligator Ck	0.008	0.482	0.594	0.131	0.717	0.376
17. <i>Gomphonema</i> cf. <i>olivaceum</i>	Alligator Ck	<0.0001	0.284	0.036	0.001	0.075	0.812
18. <i>Gomphonema</i> cf. <i>truncatum</i>	Alligator Ck	<0.0001	0.064	0.650	0.003	0.075	0.346
19. <i>Navicula</i> cf. <i>radiosa</i>	Alligator Ck	0.461	0.925	0.717	0.451	0.994	0.094
20. <i>Navicula</i> cf. <i>cryptocephala</i>	Alligator Ck	1.000	1.000	1.000	0.999	1.000	0.858
21. <i>Rhopalodia</i> cf. <i>musculus</i>	Alligator Ck	<0.0001	0.609	0.708	<0.0001	0.512	0.392
22. <i>Ulnaria</i> cf. <i>acus</i>	Alligator Ck	0.017	0.232	0.508	1.000	1.000	1.000
23. <i>Ulnaria ulna</i>	Alligator Ck	0.287	0.400	0.845	<0.0001	0.100	0.301
24. <i>Cocconeis</i> cf. <i>placentula</i>	Gowrie Ck	0.017	0.605	0.706	-	-	-
25. <i>Eunotia</i> spp.	Gowrie Ck	<0.0001	0.040	0.451	-	-	-

Taxa	Site	Atrazine			Glyphosate		
		Concentration	Light Level	Interaction	Concentration	Light Level	Interaction
26. <i>Gomphonema cf. parvulum</i>	Gowrie Ck	0.701	1.000	0.844	-	-	-
27. <i>Gomphonema sp.</i>	Gowrie Ck	0.002	0.980	0.141	-	-	-
28. <i>Navicula cf. cryptocephala</i>	Gowrie Ck	0.958	0.999	.0884	-	-	-
29. <i>Navicula cf. veneta</i>	Gowrie Ck	0.294	0.999	0.583	-	-	-
30. <i>Nitzschia sp.1.</i>	Gowrie Ck	0.116	0.126	0.135	-	-	-
31. <i>Nitzschia sp.2</i>	Gowrie Ck	0.208	0.231	0.509	-	-	-
32. <i>Ulnaria ulna</i>	Gowrie Ck	<0.0001	0.595	0.768	-	-	-
33. <i>Cymbella spp.</i>	Liverpool Ck	0.967	0.999	0.990	-	-	-
34. <i>Amphora sp.</i>	Liverpool Ck	0.415	0.430	0.712	-	-	-
35. <i>Eunotia sp.</i>	Liverpool Ck	0.015	0.999	0.001	-	-	-
36. <i>Gomphonema sp.</i>	Liverpool Ck	0.173	0.999	0.842	-	-	-
37. <i>Navicula cf. radiosa</i>	Liverpool Ck	0.919	0.034	0.421	-	-	-
38. <i>Navicula cf. germainii</i>	Liverpool Ck	0.999	1.000	0.646	-	-	-
39. <i>Navicula cf. veneta</i>	Liverpool Ck	0.277	0.001	0.020	-	-	-
40. <i>Nitzschia spp.</i>	Liverpool Ck	0.990	1.000	1.000	-	-	-
41. <i>Pinnularia sp.</i>	Liverpool Ck	0.099	1.000	0.756	-	-	-
42. <i>Ulnaria ulna</i>	Liverpool Ck	0.001	0.570	0.320	-	-	-

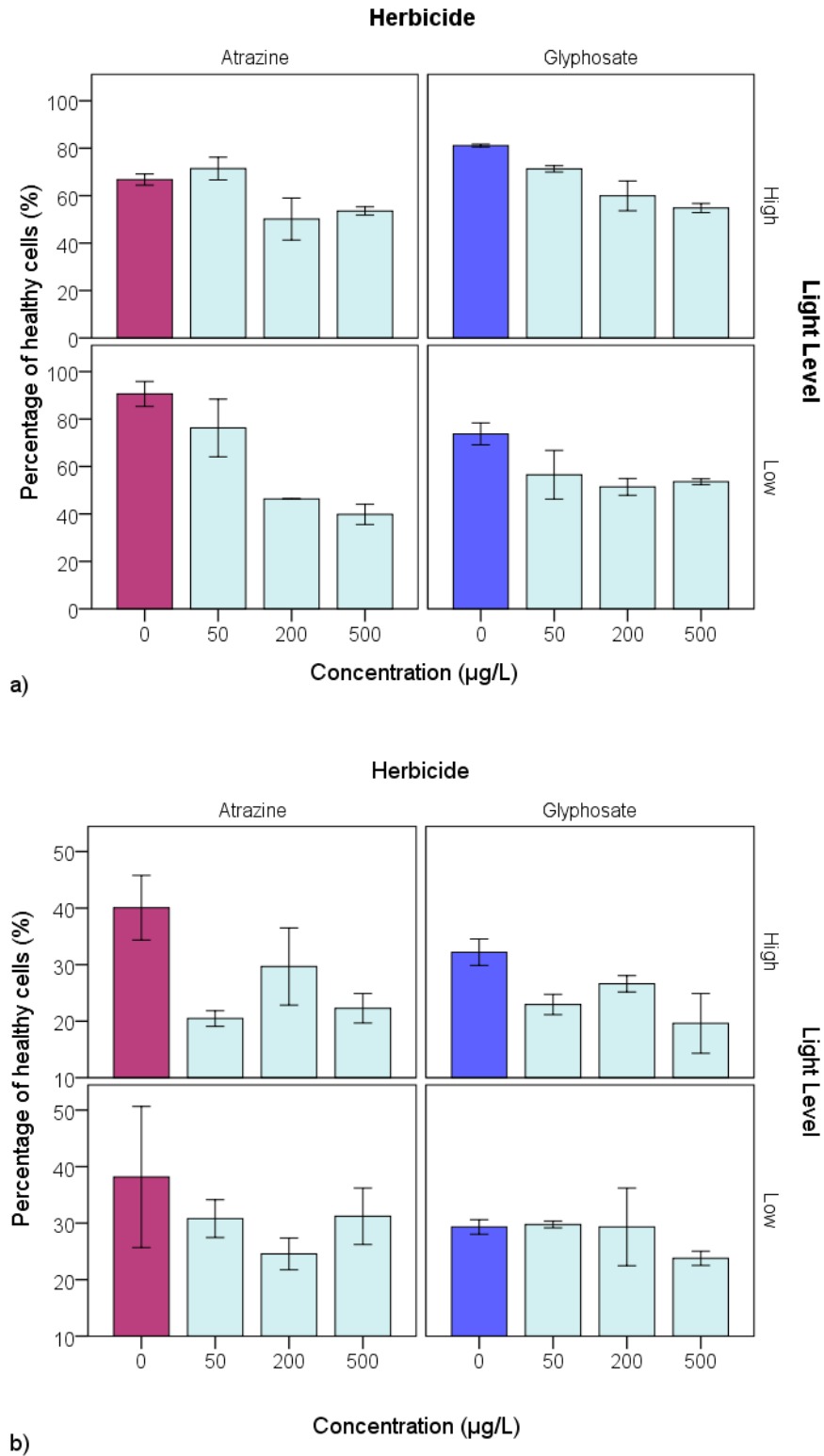


Figure 5 Histogram showing percentage of healthy cells in two taxa; a) *Gomphonema cf. olivaceum*; Alligator Ck and b) *Ulnaria sp.*; Baratta Ck, after exposure to either atrazine or glyphosate (50, 200 or 500 µg/L), at two different light levels compared to controls (purple = ethanol controls, blue = site water controls).

Relative herbicide sensitivity

Cocconeis cf. placentula, *Gomphonema cf. parvulum* and *Ulnaria ulna* showed statistically significant declines in the proportion of healthy cells with increasing herbicide exposure. For *Cocconeis cf. placentula* and *Ulnaria ulna* this was only significant within low light treatments. However in *Gomphonema cf. parvulum* the decline in the proportion of healthy cells was significant only in high light conditions. *Encyonema sp.* and *Ulnaria ulna* were the only taxa to show a dose response relationship, although this was only significant in low light conditions and at the highest concentration treatments. *Nitzschia spp.*, *Cymbella sp.* and *Gomphonema cf. parvulum* appear to respond to atrazine exposure more in low light conditions, although this is not significant. The proportion of healthy *Gomphonema cf. truncatum* cells significantly declined with Atrazine exposure under both high and low light conditions but only responded to Glyphosate under low light conditions. *Rhopalodia sp.* was sensitive to both herbicides and was less responsive to glyphosate under low light conditions. *Cymbella cf. lanceolata* appeared to respond to both Atrazine and Glyphosate exposure under both light conditions, however due to low control health the difference was not significant. *Epithemia*, *Eunotia*, *Gomphonema cf. gracile* appear to have greater responses to herbicide exposure in low light conditions however this is not significant.

Both *Ulnaria* species appeared sensitive under both light conditions however this was only significant with Atrazine exposure in low light for *Ulnaria cf. acus* and with Glyphosate exposure in high light for *Ulnaria ulna*. Both *Navicula* species were unresponsive to herbicides in either light condition, glyphosate concentration exposure and light were dependent in *Navicula cf. radiosa*. *Cymbella cf. lanceolata* was sensitive to both atrazine and glyphosate in both light conditions. *Gomphonema cf. parvulum* appeared more responsive to herbicide concentration under high light conditions, although this was not significant. *Gyrosigma sp.* only appeared responsive at high concentrations of atrazine and glyphosate, however this was not significant. *Pinnularia sp.* appeared responsive to atrazine at the highest concentration treatment level only and was not affected by increasing glyphosate exposure at all. The proportion of healthy *Melosira sp.* declined under high light conditions to glyphosate exposure, but not to Atrazine exposure. *Melosira varians* appeared to respond to Atrazine exposure, though this was not significant. The three *Navicula* species, *Navicula cf. schroeteri*, *Navicula cf. gregaria* and *Navicula cf. cryptocephala* were unresponsive to either herbicide even at the highest concentration treatments, nor at any light level.

Discussion

The results of this study indicate that light does influence the herbicide sensitivity of a few individual taxa; however it does not alter the relative sensitivity of the taxa within the benthic community. The response of benthic diatoms to increasing herbicide concentration was not light dependent for a majority of the taxa in this study. The interaction of light and herbicide concentration was significant in *Ulnaria sp.*, *Gomphonema cf. olivaceum*, *Eunotia sp.*, and *Navicula cf. veneta* (4 out of 32 taxa). For the purpose of classification of diatoms as either at risk or not at risk of herbicides, the effects of light would not alter classification in any of the taxa tested in this study. Diatoms that were most sensitive to herbicides in the

benthic community under high light exposure conditions were also the most sensitive under low light conditions, and the same was true for tolerant species.

Light intensity and herbicide concentration may interact to synergistic or antagonistic effect depending on species (Fischer et al. 2010). The current study observed differences in the response between taxa to light and herbicide concentration effects, however in the majority of cases the influence of light was insignificant. The most sensitive taxa were responsive to herbicide effects at both light levels (*Cymbella* spp., *Gomphonema* cf. *olivaceum*, *Rhopalodia* cf. *musculus*), with some sensitive taxa response appearing greatest in low light treatments (*Cymbella* cf. *lanceolata*, *Gomphonema* cf. *truncatum*, *Encyonema* sp., *Ulnaria* ulna). Some of the more tolerant species also appeared to be more responsive at low light levels however an interaction between light and concentration treatments was not significant (*Epithemia*, *Eunotia* cf. *minor*, *Cocconeis* sp., *Cocconies* cf. *placentula*, *Melosira* *varians*, *Pinnularia* sp. *Nitzschia* spp.). The health of the most tolerant taxa did not decline at any light level to either herbicide even at the highest concentration treatments (*Eunotia* spp., *Gyrosigma* sp., *Navicula* cf. *schroeterii*, *Navicula* cf. *rostellata*, *Navicula* cf. *cryptocephala* and *Navicula* cf. *veneta*).

Conclusions

Light conditions during exposure did not alter the effects of herbicide on cell health in the majority of taxa in this study. In no cases did sensitive species appear to be tolerant, or *vice versa*, under altered light conditions. The sensitivity data produced supports the suitability of benthic diatoms as indicators of herbicide toxicity in rivers.

Chapter 4 – Classification of diatoms from rivers draining on to the GBR as at risk or not at risk from herbicides (SPEAR_{herbicides})

Diatom taxa collected during our field surveys and during the toxicity tests were assigned as SPEcies At Risk (SPEAR) or SPEcies not At Risk (SPEnotAR) based on their sensitivity to herbicides as determined in the aforementioned rapid toxicity tests or from toxicity test results in the scientific literature. Observations showed minimal variation in the classification of tested species within diatom genera but variation between related genera so the classification was not extrapolated beyond genera and the classification is referred to as SPEAR_{genus} (Appendix 4). This has resulted in a number of genera which we have no data on their sensitivity to herbicide and thus are not classified (indicated by -999 in Appendix 4).

Unlike the macroinvertebrate based SPEAR classification, the preliminary classification of the diatoms was only based on their physiological sensitivity to herbicides and did not include recoverability or avoidance traits (i.e. ecological sensitivity) due to lack of information on these traits.

Chapter 5 - Assigning of traits to stream macroinvertebrates

A list of all stream macroinvertebrate taxa previously collected by the Queensland Department of Science, Information Technology, Innovation and the Arts (DSITIA) and its predecessors from river catchments that drain on the GBR was compiled. The taxa on this list were match with an existing trait database for SPEAR_{pesticides} in Victoria and South Australia (Schäfer et al. 2011a). Then a workshop was held in Brisbane to review the relevance of the compiled traits for north Queensland and to assign traits to north Queensland taxa not present in Victoria or South Australia. The workshop participants were Ben Kefford (University of Technology, Sydney), Jane Hughes (Griffith University), Richard Pearson (James Cook University) and Satish Choy, Jason Dunlop and Raj Prasad Alisha Steward and Jonathan Marshall (DSITIA).

Assigning of traits to stream macroinvertebrates

The full list of traits assigned to stream macroinvertebrates is presented in Appendix 2 and references are provided in Appendix 3. While there were many cases of taxa from north Queensland taxa that were judged to have different traits than those in Victoria and South Australia, only in 4 cases (of 169 taxa classified) did this result in different in SPEAR classification (Table 18).

Table 4. Stream macroinvertebrate taxa with discordant SPEAR/SPENotAR from pesticides classification in Victoria/South Australia and north Queensland. Trait information, generation (G) assessment, dispersal assessment and out of water assessment below are for north Queensland. Full list of taxa from north Queensland and their trait information and references is provided in Appendix 2 & 3.

Lowest ID in Qld database	Order	Sorg	S assessment	SPEAR assessment Vic & SA	SPEAR assessment nQLD	Number of generations per year	Time until reproduction (a)	G assessment	Dispersal capacity	Dispersal assessment	Duration of life stages out of water	out of water assessment
Atyidae	Decapoda	-0.08	1	0	1	1-2	0.75-1.5	1	high, Paratya - low, Caradina - high, Australatya - mod/high	0	fully aquatic	1
Caenidae	Ephemeroptera	-0.3	1	0	1	1-3	0.5-1	1	high-mod	0	short	1
Diptera	Diptera	-0.35	1	1	0	1-many	0.5	0		1	short	1
Dixidae	Diptera	-0.35	1	1	0	>2	0.5	0		1	short	1

Chapter 6 - Relationships between $SPEAR_{pesticides}$ and $SPEAR_{herbicides}$ and measured pesticides

Stream macroinvertebrate and benthic diatom samples were collected from DSITIA's 11 river pesticide monitoring sites that are part of the Great Barrier Reef Catchment Loads Monitoring Program and from 3 reference sites before and after both of the 2011/12 and 2012/13 wet seasons. (For the sampling before the 2011/12 season, it was unsafe to sample one site due to high crocodile risk and it was thus not sampled).

Pesticide data

Pesticide data from the 2011/12 wet-season is currently (Oct 2013) available but similar data from the 2012/13 wet-season is not available, so we have only considered relationships between $SPEAR$ indexes and measured pesticide from the 2011/12 wet-season.

Diatom-based $SPEAR_{diatoms}$

The preliminary diatom-based $SPEAR_{herbicides}$ index after the 2011/12 wet-season at pesticide monitoring sites were negatively correlated with the $\log_{10}$ transformed 95th percentile of TEQ_{so} in the 2011/12 wet-seasons (Figure 6). This indicates that as the peak measured concentration of photosystem II inhibitors (PSII) increases, there is a decrease in the abundance of herbicide sensitive diatom species.

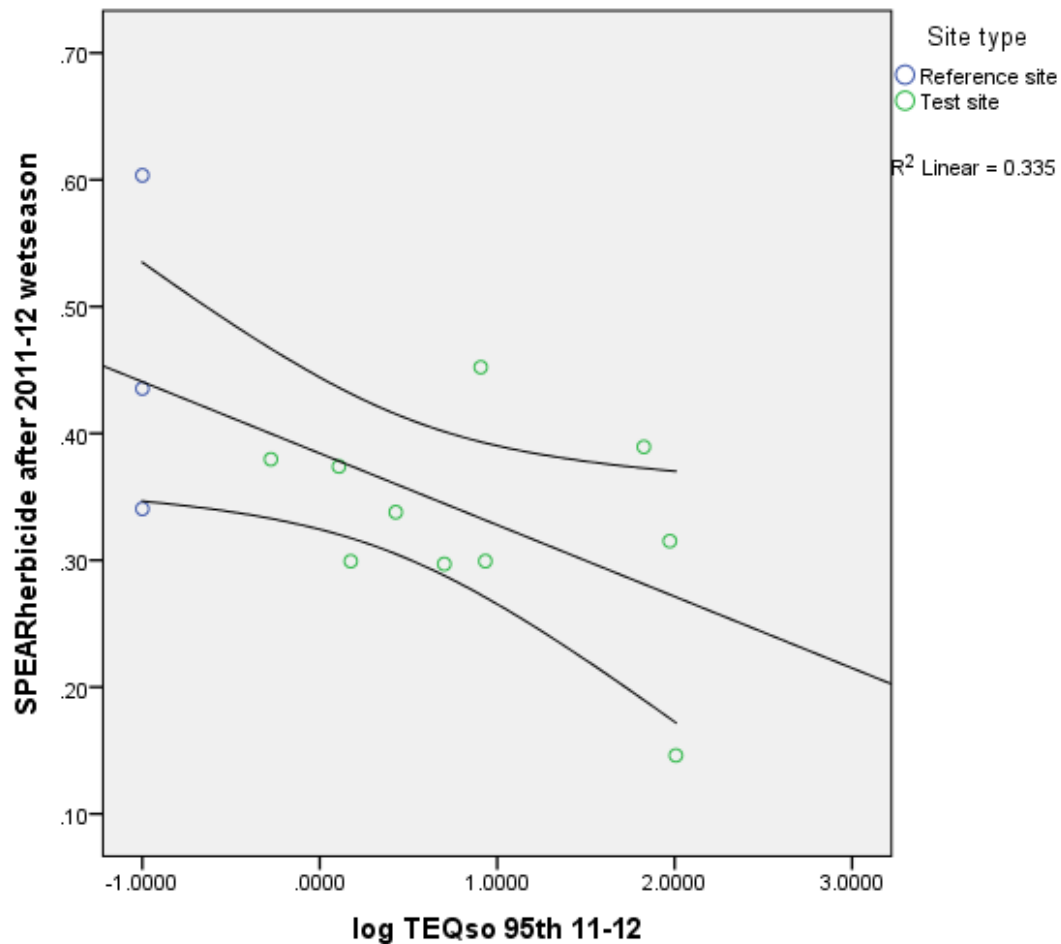


Figure 6. The relationship between the diatom-based SPEARherbicides after the 2011/12 wet-season and the 95th percentiles atrazine toxic Equivalent concentrations (TEQ) concentrations calculated at sites monitored for pesticides in 2011/12. Reference sites were assumed to have -1 log TEQso (to be lower the least polluted pesticide monitoring site) TEQso data supplied by Rachael Smith and Michael Warne, DSITIA.

There were no relationships between the SPEAR_{herbicides} index prior to the 2011/12 (Figure 54) and 2012/13 (Figure 55) wet-seasons and the measured herbicides levels. This result suggests that the diatoms flora recovers from the effect observed at the end of the wet season (Figure 52).

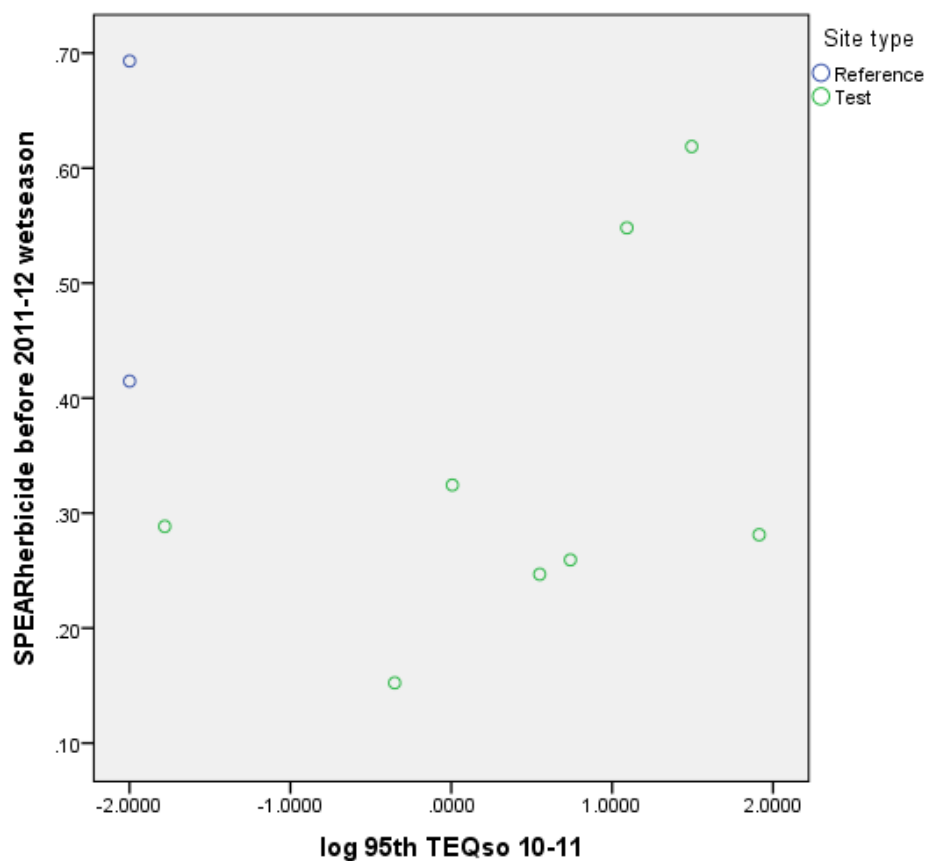


Figure 7. The relationship between the diatom-based SPEARherbicides before the 2011/12 and the 95th percentiles atrazine toxic Equivalent concentrations (TEQ) concentrations in the preceding wet-season (2010/11). Reference sites are assumed to have -2 log TEQso (to be lower the least polluted pesticide monitoring site). TEQso data supplied by Rachael Smith and Michael Warne, DSITIA.

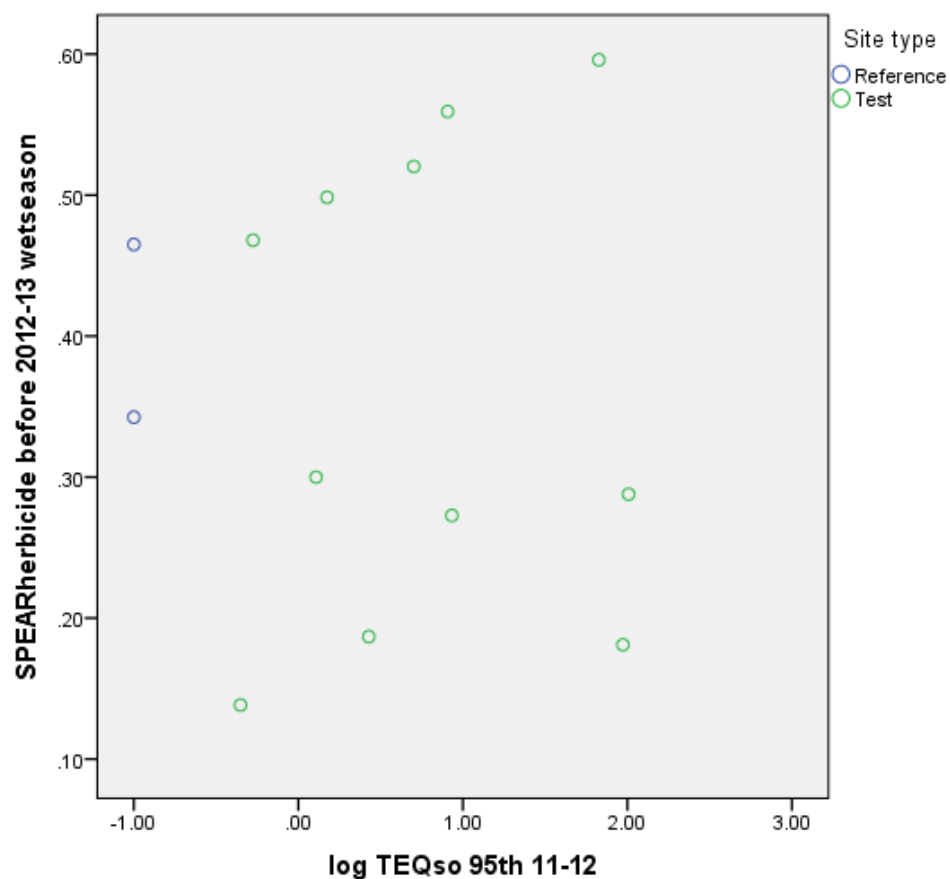


Figure 8. The relationship between the diatom-based SPEARherbicides before the 2012/13 and the 95th percentiles atrazine toxic Equivalent concentrations (TEQ) concentrations in the preceding wet-season (2011/12). Reference sites are assumed to have -1 log TEQso (to be lower the least polluted pesticide monitoring site). TEQso data supplied by Rachael Smith and Michael Warne, DSITIA.

Macroinvertebrate base $SPEAR_{pesticides}$

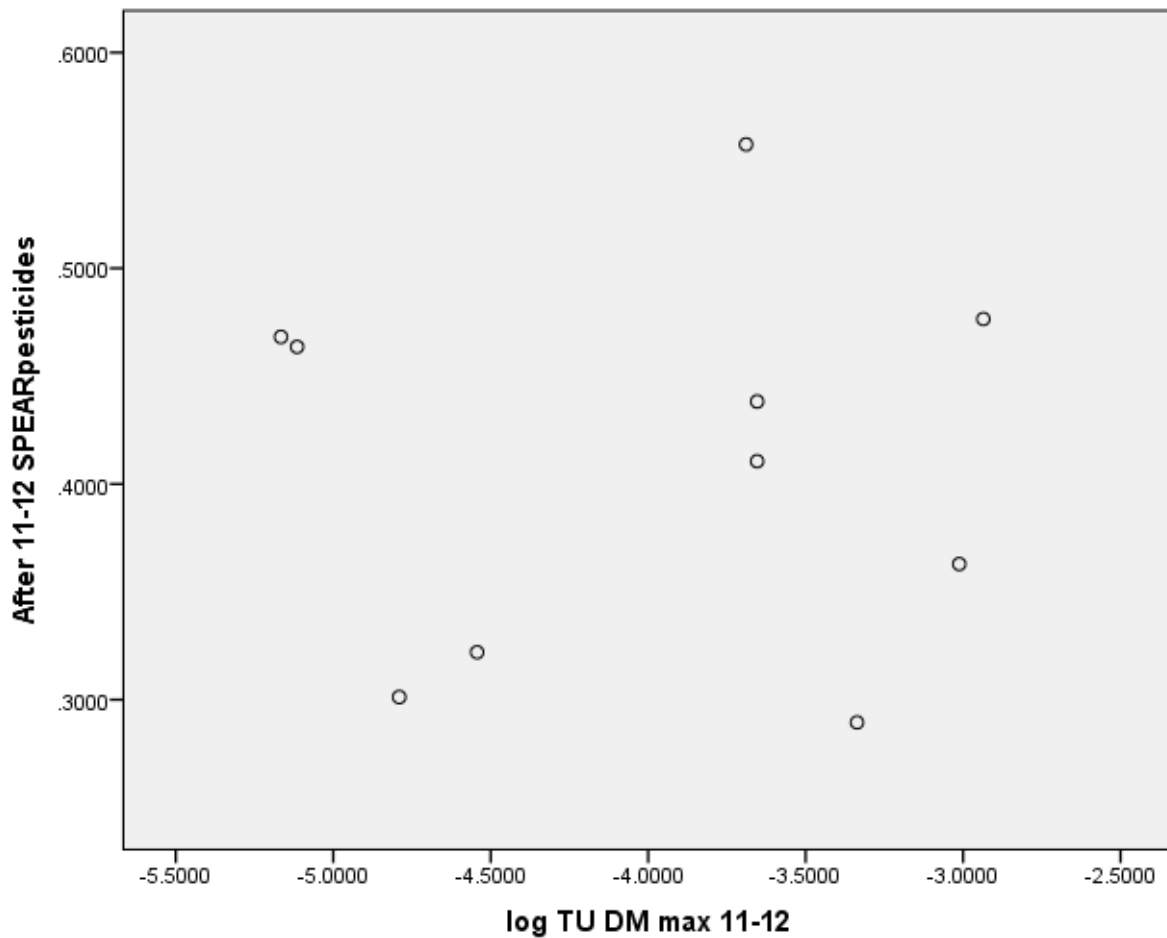


Figure 9. The relationship between the macroinvertebrate-based $SPEAR_{pesticides}$ after the 2012/13 and maximum toxic units to the standard test organism *Daphnia magna* (TU DM max) a crustacean in the 2010/11 wet-season. Pesticide data supplied by Rachael Smith and Michael Warne, DSITIA.

There was no relationship between the macroinvertebrate $SPEAR_{pesticides}$ after the 2011/12 wet-season and the measured level of pesticide toxicity - in terms of the maximum log toxic units to model invertebrate species *Daphnia magna* (log TU DM max) – (Figure 55). However considering the toxicity of the chemical detected to *D. magna* were low (mostly less than log -3 TU DM or 1/1000th of *D. magna*'s 48h LC50) in both the 2011/12 and the 2010/11 wet-seasons (Figure 56) it would appear unlikely that pesticides would be affecting the stream macroinvertebrate communities (Schäfer et al. 2012). The one exception was the Tully River (at Davidson Rd at Euramo) where in 2010/11 wet-season the log TU DM was -0.36 as a result of Permethrin, indicating the measured concentration of was 44% of *D. magna*'s 48h LC50. Thus at this site at in 2010/11 effects on the macroinvertebrate community would be likely but as biotic sampling did not occur in this wet-season, they cannot be confirmed.

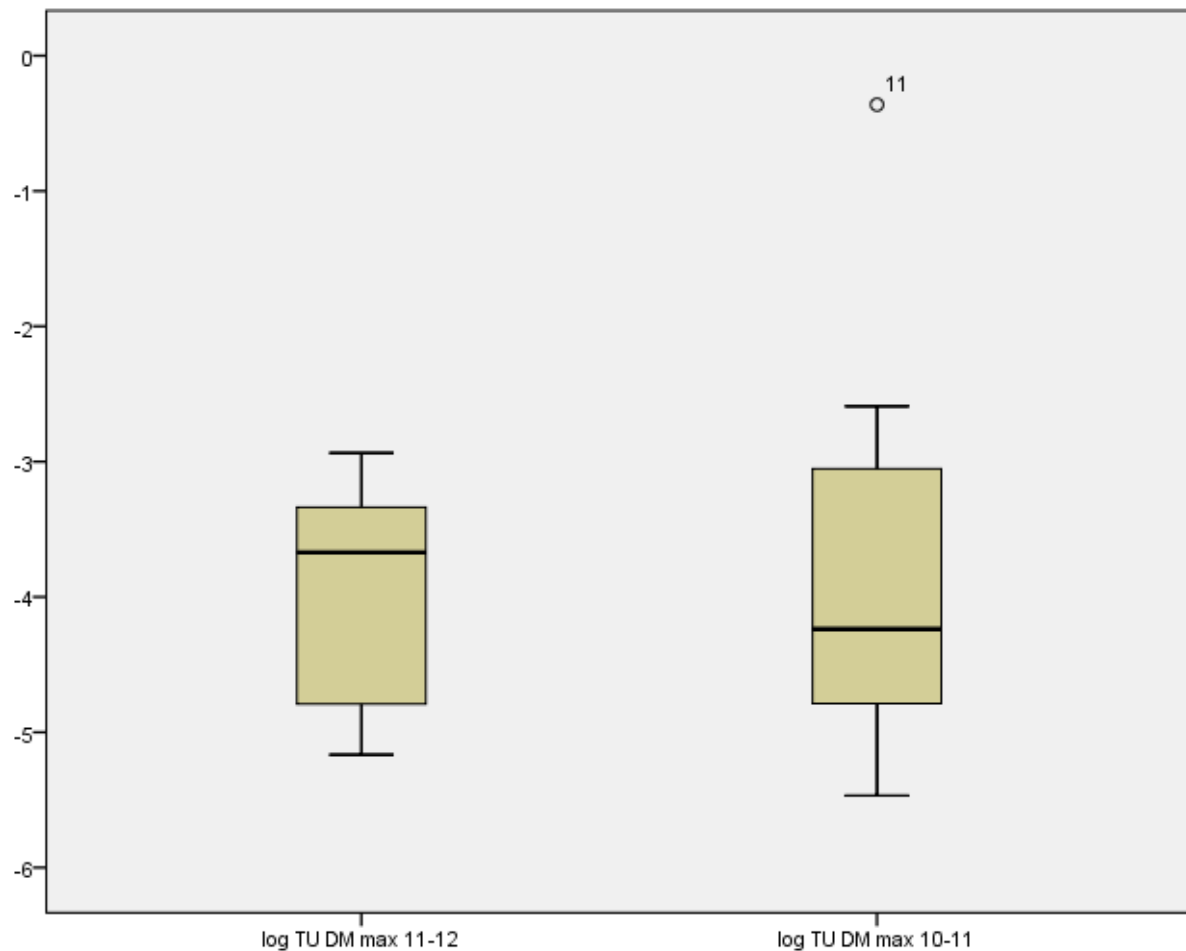


Figure 10. Box plots of measured concentrations expressed in terms of maximum toxic units to the standard test organism *Daphnia magna* (TU DM max) a crustacean in the 2011/10 and 2010/11 wet-seasons. Boxes show 25th and 75th percentiles, with the line in the middle of the box the 50th percentile (median). The whiskers show either the minimum and maximum values or 1.5 times the height of the box (whichever is smaller). The circle is an outlier (defined as located outside the box by >1.5 but ≤ 3 times the height of the box). Pesticide data supplied by Rachael Smith and Michael Warne, DSITIA.

Discussion

Relationships between $SPEAR_{pesticides}$ and $SPEAR_{diatoms}$ and measured pesticides

The diatom based $SPEAR_{diatoms}$ at the pesticide monitoring sites after the 2011/12 wet season was correlated with the concentration of photosynthesis II inhibiting herbicides measured in that wet-season expressed in terms of atrazine toxicity Equivalent concentrations (TEQso).

It is not surprising that there was only a relationship between $SPEAR_{herbicides}$ and herbicide toxicity after a wet-season and not immediately before two wet-seasons. Freshwater diatoms have relatively short life-cycles and thus they tend to be relatively resilient following disturbances. Sampling of the diatom flora throughout a year, could clarify if this is indeed the case. In the meantime it is important that any use of $SPEAR_{herbicides}$ as a monitoring tool include sampling of diatoms immediately following the wet-season.

When pesticide data is available for the 2012/13 wet-season, we will determine if the relationship between $SPEAR_{herbicides}$ and TEQso observed in 2011/12 is repeated after the following wet-season.

A definitive test of whether $SPEAR_{herbicides}$ really responds to herbicides and only herbicides could be conducted using experimental mesocosm experiments. Such studies would have water flowing through or re-circulating (by means of a pump) multiple mesocosm (or artificial) streams. Such experiments would involve experimentally adding herbicides to some mesocosm stream, altering other environmental factors that are confounded with herbicide toxicity (or suspected might alter $SPEAR_{herbicides}$) to other stream and having additional streams as controls (i.e. changing nothing). By recording the response of the diatom flora in general and the $SPEAR_{herbicides}$ index in particular in these various treatments could determine if $SPEAR_{herbicides}$ really is responding to herbicides and to only herbicides.

At the pesticide monitoring sites, the concentrations of pesticides measured during the 2011/12 wet-season did not reach levels that are likely to have detectable effects on stream macroinvertebrate communities. Chemical measurements indicate that Tully River @ Euramo in the preceding wet-season (2010/11) did reach concentrations that were likely to impact stream invertebrates but there was no concurrent biotic sampling. Consequently it is not surprising that there was no relationship between the level of pesticide toxicity to the standard organism *D. magna* and the macroinvertebrate-based $SPEAR_{pesticides}$. This suggests that at the 11 pesticides monitoring and three reference sites the concentrations of pesticides were not at levels that are directly detrimental to freshwater invertebrates.

It is worth noting that the measurements of pesticides that are most likely to affect stream macroinvertebrates is complicated by some technical considerations. The chemical monitoring program employed two methods of quantification of the chemical concentrations of pesticides depending on the specific properties of the individual pesticides. These methods were high performance liquid chromatography–mass spectrometry (HLCMS) which was used mainly to quantify the concentrations of herbicides

(in particularly: Ametryn, Atrazine Bromacil, Desethyl Atrazine, Desisopropyl Atrazine, Diuron, Fluometuron, Hexazinone, Metolachlor, Prometryn, Simazine, Tebuthiuron and Terbutryn all with a reporting limit of 0.01 µg/L) and one insecticide (Imidacloprid) and thus this method is of less relevance for macroinvertebrates as they are generally relatively tolerant of herbicides compared to photosynthetic organisms. The second method, gas chromatography-mass spectrometry (GC-MS) was used to quantify the concentration of 109 compounds (Appendix 5) including a wide range of insecticides and fungicides and thus GC-MS is of more relevance. HLCMS was employed on all water samples analyzed for pesticides but GC-MS was only used on a small subset of the samples analyzed by HLCMS, which opens the possibility that peak concentrations of chemical quantified by GC-MS were underestimated or falsely not detected (relative to those chemicals measured by HLCMS). So the most relevant method for quantifying pesticides most likely to affect stream macroinvertebrates (GC-MS), likely underestimates the concentrations of chemicals and did not detect other chemicals which are potentially present.

Conclusions/Future Directions

Toxicity testing of diatoms

The rapid toxicity testing of diatoms was able to establish a classification of diatom taxa in terms of their herbicide sensitivity. The experiments conducted show that this classification is independent of the type of herbicides, the level of light, nutrient levels and whether the diatoms were collected from a site with known herbicide pollution or a reference site. These findings gave confidence that the diatom flora could make a useful tool for the biomonitoring of herbicide contamination. The classification appears not to be extrapolation beyond the level of genus and thus there were a number of genera which were not classified (Appendix 3). Future studies should target these genera for toxicity testing.

The diatom base SPEAR_{herbicides}

After the 2011/12 wet-season, the diatom-based SPEAR_{herbicides} index at the pesticide monitoring sites was correlated with the peak levels (95th percentile) of photosystem II inhibitors (PSII) herbicides expressed in terms of atrazine TEQ_{so} measured in that wet-season. When pesticide data is available for the 2012/13 wet-season we will determine if a correlation exists between SPEAR_{herbicides} and TEQ_{so} measured in the following wet-season.

Prior to the 2011/12 and 2012/13 wet seasons, the diatom flora appeared to have recovered over the 2011 and 2012 dry-seasons, respectively, from herbicides impacts during the preceding wet-season.

The macroinvertebrate-based SPEAR_{pesticides}

The values of the macroinvertebrate-based SPEAR_{pesticides} observed at the 11 pesticide monitoring sites and the concentrations of pesticides most toxic to invertebrates suggest that at these sites in 2011/12 there was not significant toxicity from pesticides on stream macroinvertebrates.

Future directions

We strongly recommend that SPEAR_{herbicides} and SPEAR_{pesticides} are determined at the pesticide monitoring sites before and after subsequent wet-seasons and used as a biomonitoring tool to add value and confidence in the findings of the chemical monitoring program. Consideration should be given to measuring these indexes at a greater number of sites on rivers draining into the GBRWHA to provide greater spatial coverage at relatively modest additional costs.

Research needs

There is still research needed to increase the confidence in the SPEAR indexes. In particular, mesocosms experiments could establish causality between the response of the SPEAR_{herbicides} and SPEAR_{pesticides} indexes and herbicides and insecticides, respectively.

Mesocosm experiments could also establish whether factors confound $\text{SPEAR}_{\text{herbicides}}$ and $\text{SPEAR}_{\text{pesticides}}$ indexes. Seasonal sampling of the diatom flora at selected sites could establish seasonal trends in $\text{SPEAR}_{\text{herbicides}}$ and to show how quickly the diatom flora recovers at polluted sites following the ending of significant pesticide pollution. It would also be useful to conduct toxicity tests with further diatom genera.

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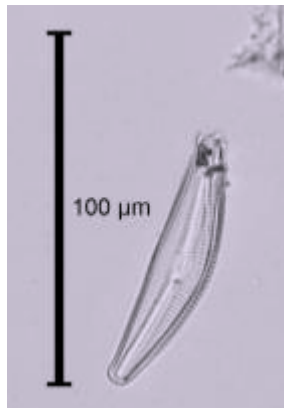
Appendix 1. Health classification of diatoms

Diatoms from Alligator Creek

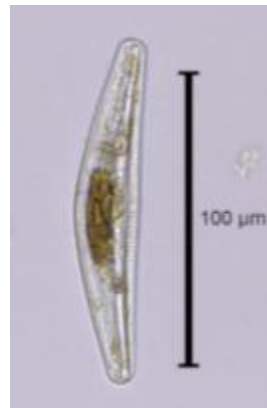
Cymbella cf. lanceolata



Healthy cell

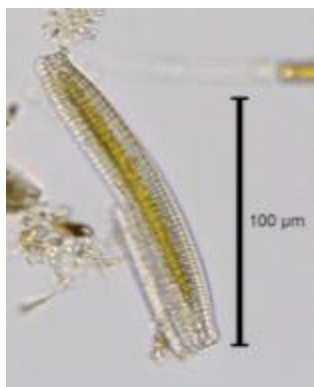


Unhealthy empty cell



Unhealthy cell with
abnormal chloroplast

Epithemia sp.

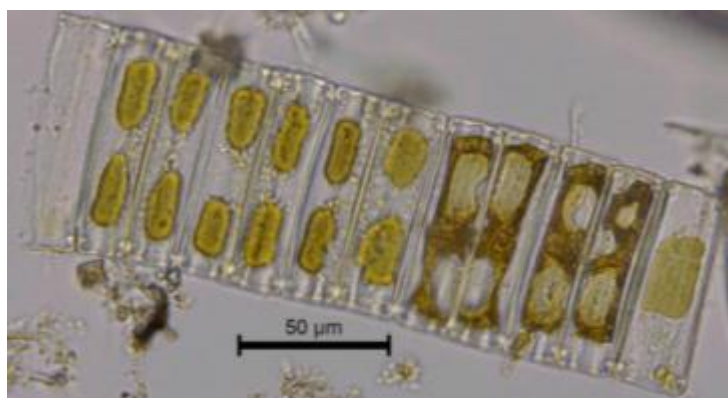


Healthy cell



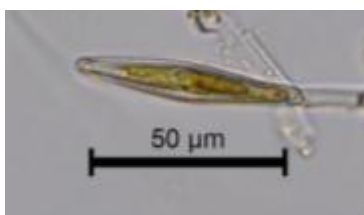
Unhealthy empty cell

Eunotia cf. minor

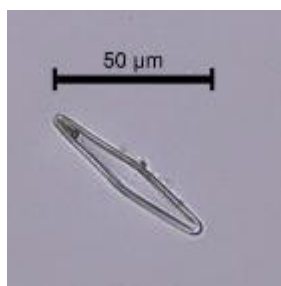


A chain of both unhealthy and healthy cells

Gomphonema cf. gracile



Healthy cell



Unhealthy empty cell



Unhealthy cell with
abnormal chloroplast

Gomphonema cf. olivaceum

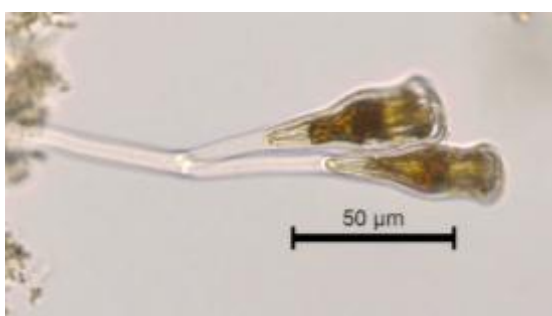


Healthy cell

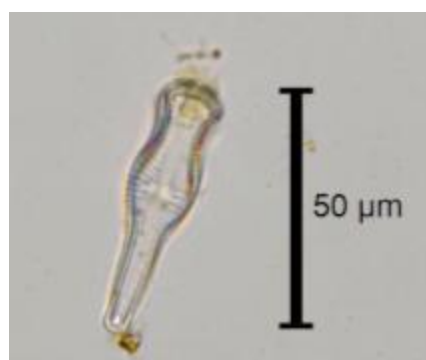


Unhealthy cell with
abnormal chloroplast

Gomphonema cf. truncatum

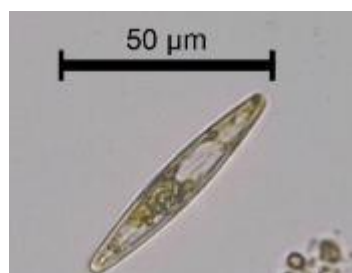
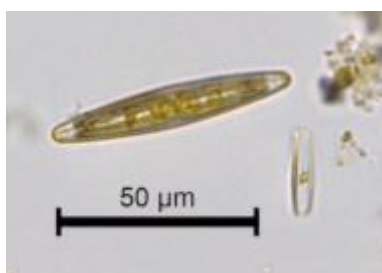


Healthy cell



Unhealthy empty cell

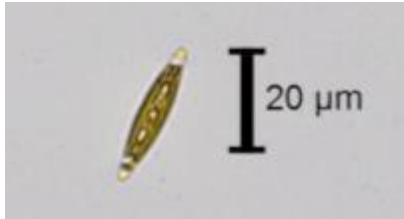
Navicula cf. radiosa



Healthy cell

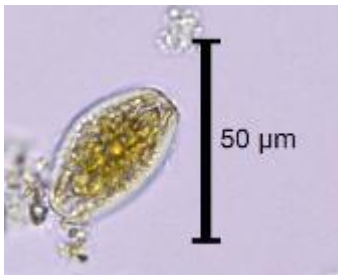
Unhealthy cell with abnormal chloroplast

Navicula cf. cryptocephala

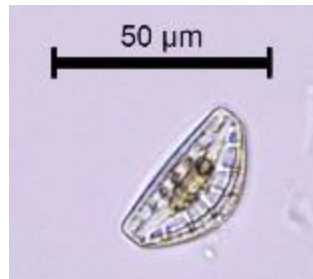


Healthy cell

Rhaplovidia cf. musculus

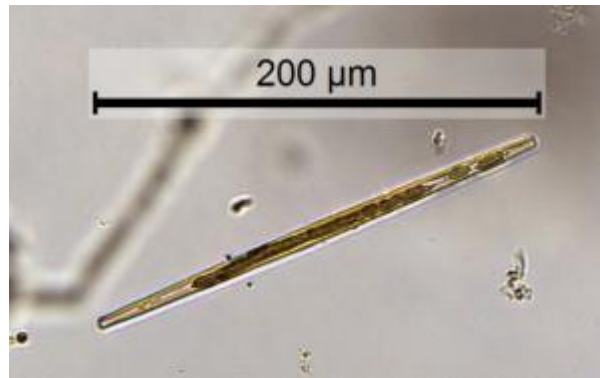
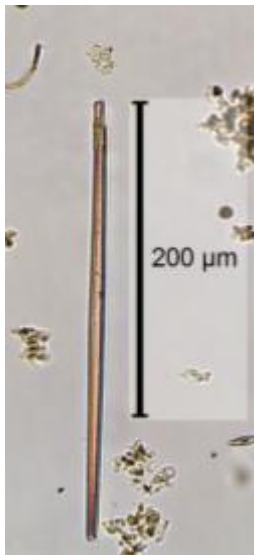


Healthy cell

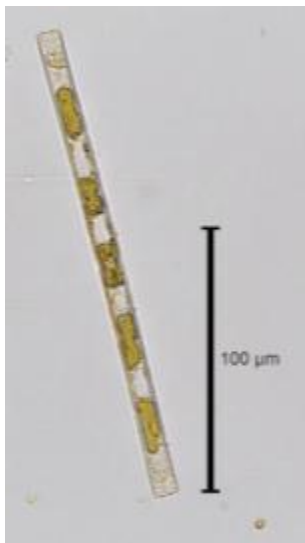


Unhealthy cell with abnormal chloroplast

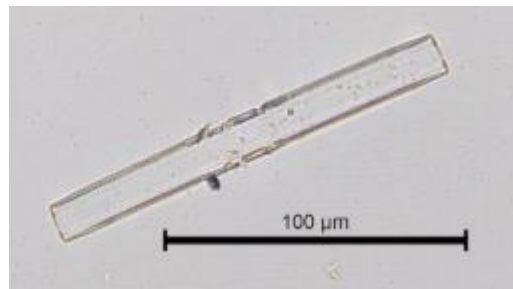
Ulnaria acus



Ulnaria ulna



Healthy cell



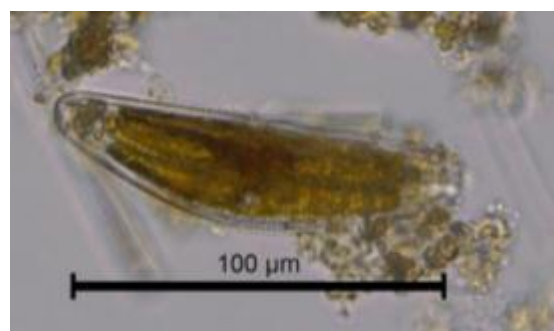
Unhealthy empty cell

Diatoms from Barratta Creek

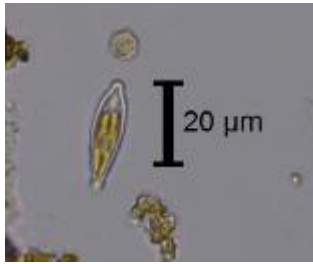
Cocconies sp.



Cymbella cf. *lanceolata*



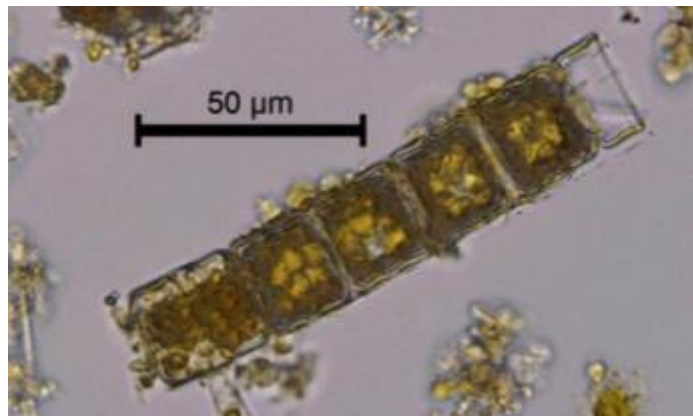
Gomphonema cf. parvulum



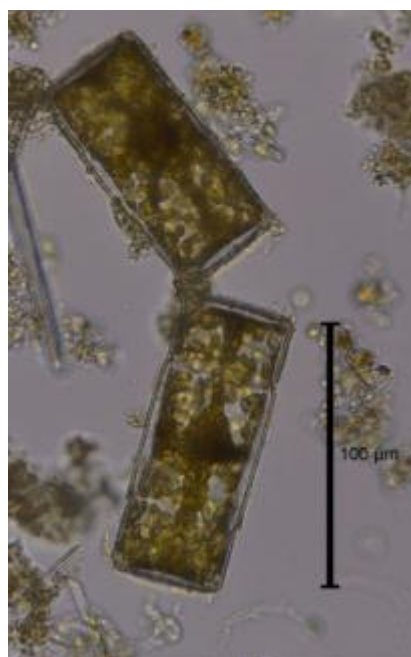
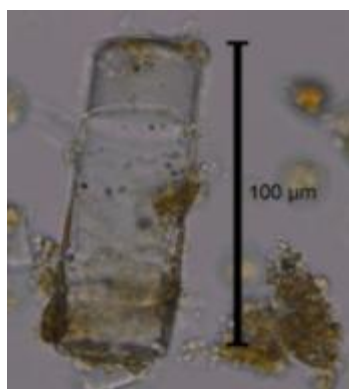
Gyrosigma



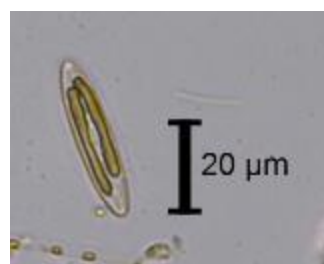
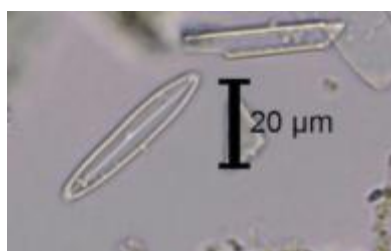
Melosira varians



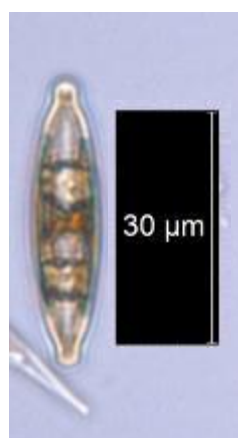
Melosira sp.



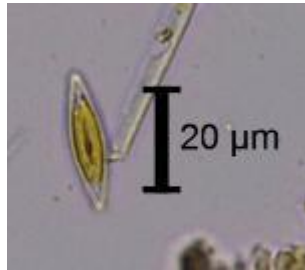
Navicula cf. *shroerti*



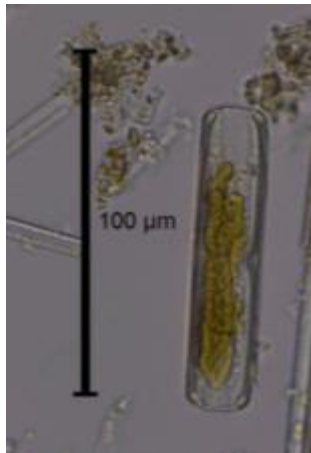
Navicula cf. *rostellata*



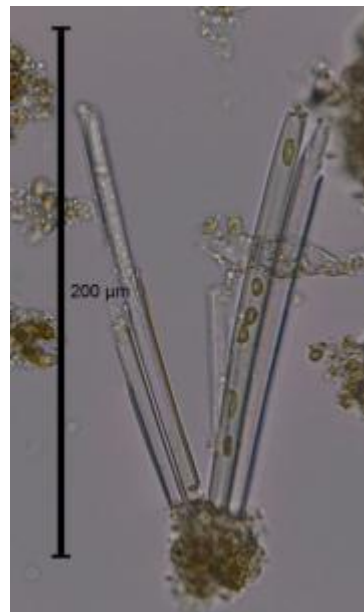
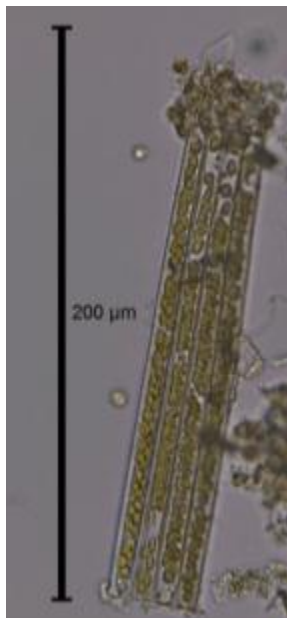
Navicula cf. veneta



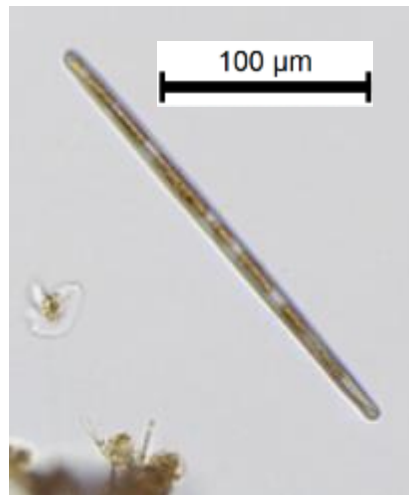
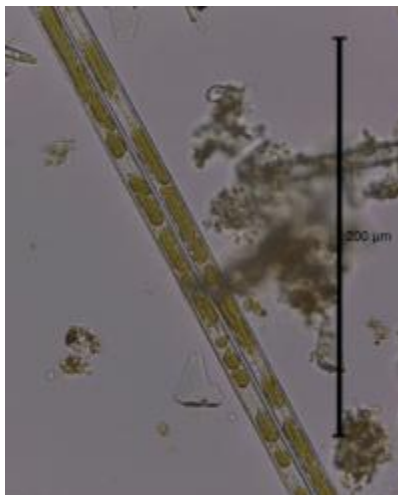
Pinnularia sp.



Ulnaria sp.

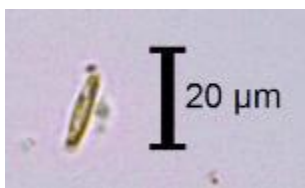


Ulnaria ulna

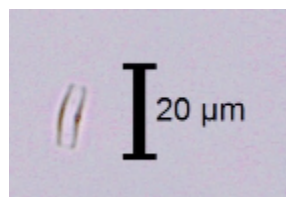


Diatoms from Bluewater Creek

Achnanthisidium

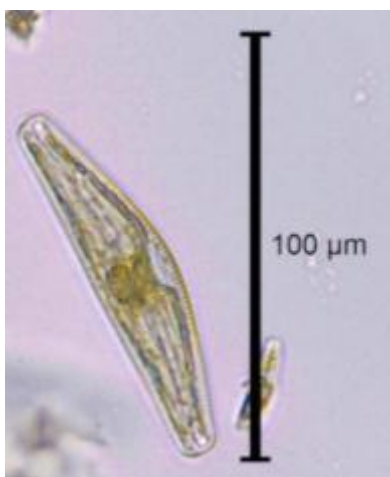


Healthy cell



Unhealthy empty cell

Cymbella



Healthy cell

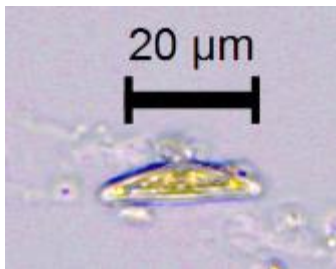


Unhealthy cell with abnormal chloroplast

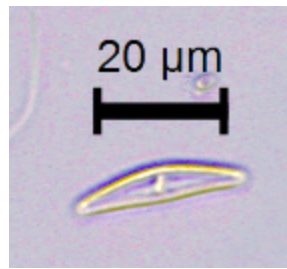


Unhealthy empty cell

Amphora

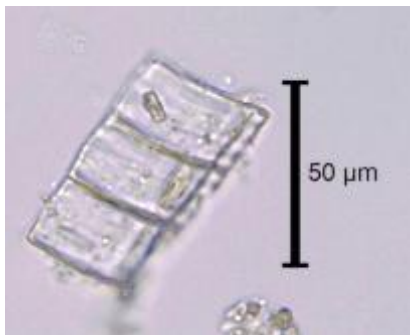


Healthy cell



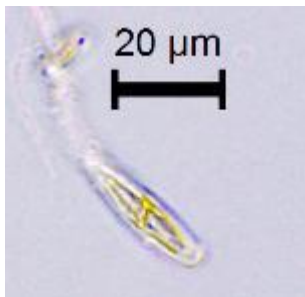
Unhealthy empty cell

Eunotia

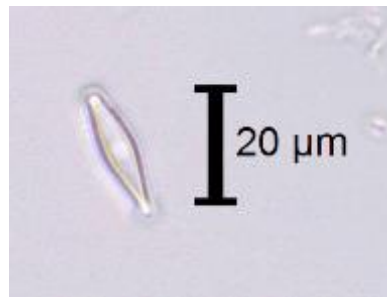


Unhealthy empty cells

Gomphonema cf. angustatum

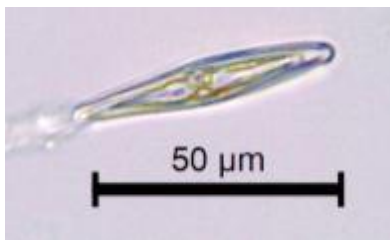


Healthy cell

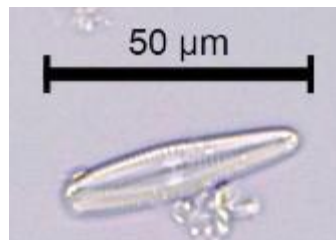


Unhealthy empty cell

Gomphonema cf. lanceolatum

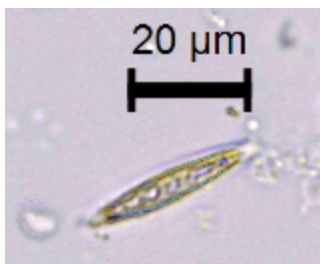


Healthy cell



Unhealthy empty cell

Navicula



Healthy cell

Pinnularia

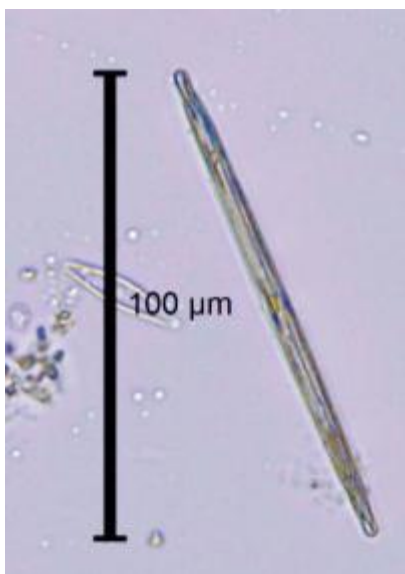


Healthy cell

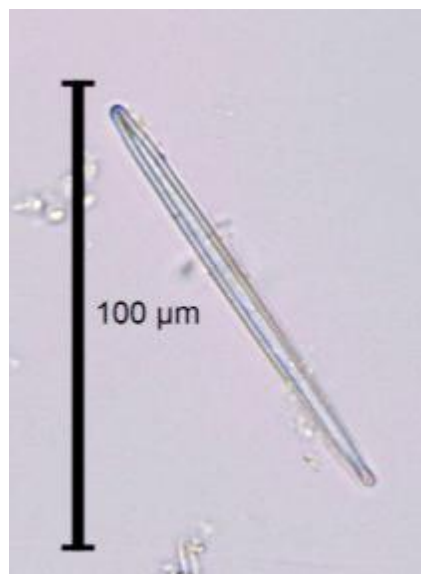


Unhealthy empty cell

Ulnaria ulna



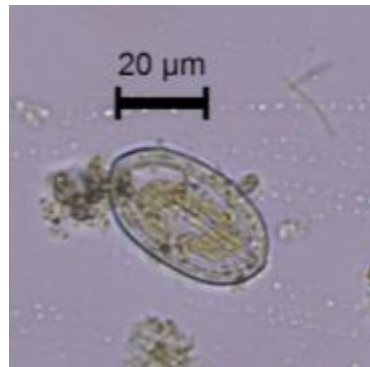
Healthy cell



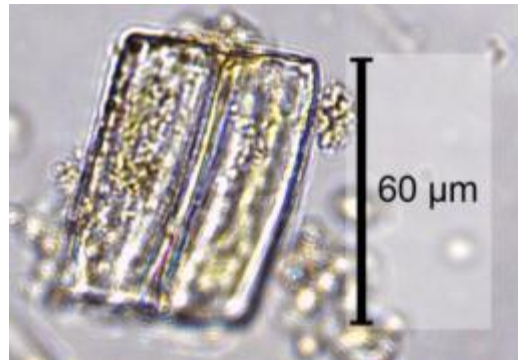
Unhealthy empty cell

Diatoms from Gowrie Creek

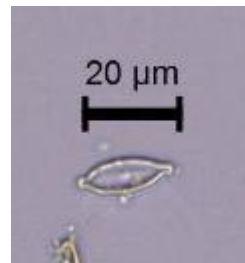
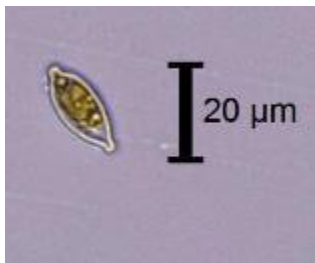
Cocconeis cf. placentula



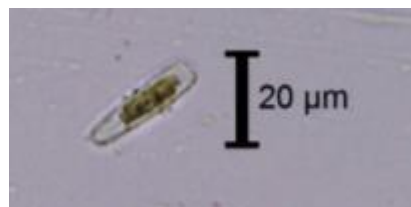
Eunotia spp.



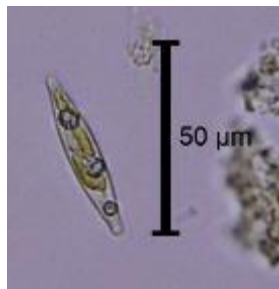
Gomphonema cf. parvulum



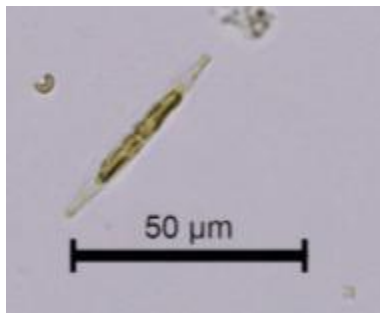
Gomphonema sp.



Navicula cf. cryptocephala



Nitzschia spp.

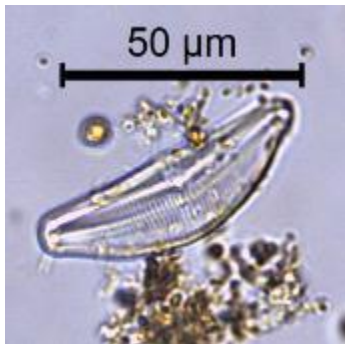


Ulnaria ulna

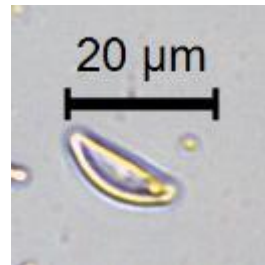


Diatoms from Liverpool Creek

Cymbella spp.



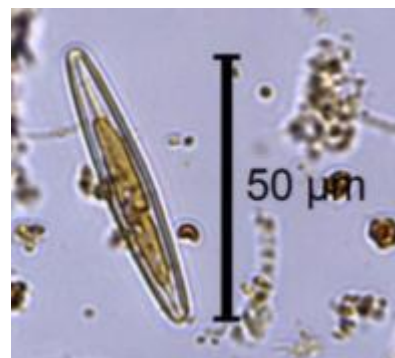
Amphora sp.



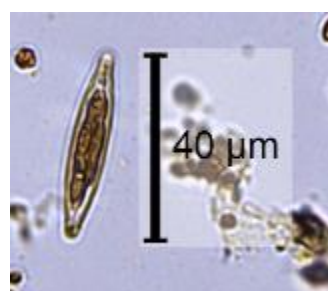
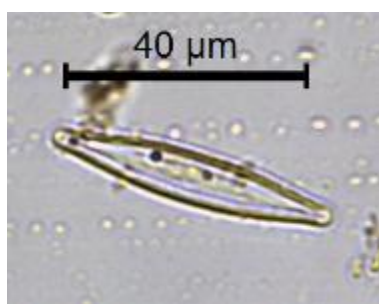
Gomphonema sp.



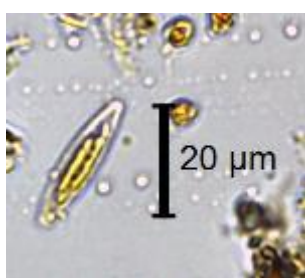
Navicula cf. *radiosa*



Navicula cf. germanii



Navicula cf. veneta



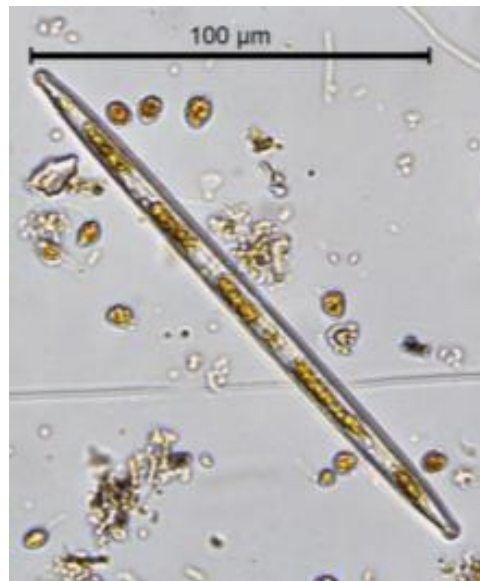
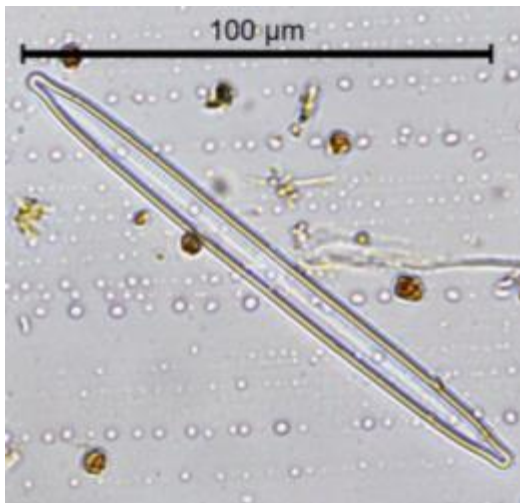
Nitzschia spp.



Pinnularia sp.



Ulnaria ulna



Appendix 2. Traits relevant for SPEARpesticides for all stream macroinvertebrates in catchments draining on to the GBR. References are given in appendix 2.

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	SPEAR assessment nQLD	Number of generations per year	Ref no	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
Acariformes	Acariformes	NULL	NULL	1		0.38	1	0	0	2-10	73	0.1-0.75	73	0	strong drifting	46	0	fully aquatic	RS	1
Aeshnidae	Odonata	Aeshnidae	NULL	1		-0.96	0	0	0	0.5-1	iorder	1	iorder	1			1	few weeks to 2-3 months (adults)	iorder	1
Ameletopsidae	Ephemeroptera	Ameletopsidae	NULL	1		-0.3	1	1	1	1-2	49	0.5-2	49	1			1	short	8:3	1
Ancylidae	Basommatophora	Ancylidae	NULL	1		-1.16	0	0	0	1-2	73	0.3	73	1			1	fully aquatic	RS	1
Anisoptera	Odonata	Anisoptera	NULL	1		-0.96	0	0	0					1			1	few weeks to 2-3 months (adults)		1
Aphelocheiridae	Hemiptera	Aphelocheiridae	NULL	1		-0.56	0	0	0	1	40	0.5	40	1			1	fully aquatic	2:213	1
Aphroteniini	Diptera	Chironomidae	Aphroteniini	1		-	0	0	0	1-4	2:246	0.2	37:79	0	strong	5; 43	0	few days	37:7	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no	Time until reproduction (a)	Ref time until repoduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
ae		e	niinae			0.39								drifting			to weeks	9		
Aphroteniinae	Diptera	Chironomidae	Aphroteniinae	1		-0.39	0	0	0	1-4	2:246	0.2	37:79	0	strong drifting	5; 43	0	few days to weeks	37:79	1
Arthropoda	NULL	NULL	NULL	1	0				0											
Athericidae	Diptera	Athericidae	NULL	1		-0.35	1	1	1	1-2	EK, QEK	0.75	EK	1			1	short	EK	1
Atriplectidae	Trichoptera	Atriplectididae	NULL	1		-0.06	1	1	1	1	EK	1	EK	1			1	1-2 months	EK	1
Atyidae	Decapoda	Atyidae	NULL	1		-0.08	1	0	1	1-2	2:164	0.75-1.5	56	1	high, Paratya - low, Caradina - high, Australatya - mod/high	QEK	0	fully aquatic	RS	1
Austrocorduliidae	Odonata	Austrocorduliidae	NULL	1		-0.96	0	0	0	1	EK	1	EK	1			1	few weeks to 2-3 months(adults)	3:1145; 71	1
Baetidae	Ephemeroptera	Baetidae	NULL	1		0.02	1	1	1	1-3	7, 45	0.5-1	8:79; 9:152	1	some strong drifting	5; 44;	1	short (November to	8:3	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment		
														taxa (Baetis sp.; Nouisa sp.); flight more important?	67		March)				
Belostomatidae	Hemiptera	Belostomatidae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0		QEK	1	Can fly	2:213, QEK	1	
Bithyniidae	Neotaenioglossa	Bithyniidae	NULL	1		-1.81777	0	0	0	1				1	low	QEK	1			1	
Bivalvia	NULL	NULL	NULL	1		-2.09	0	0	0	1-2	QEK	0.2	iorder	1	low	3:1243	1	fully aquatic	RS	1	
Blephariceridae	Diptera	Blephariceridae	NULL	1		-0.35	1	1	1	1-2	3:1192	0.5-0.75	3:1192	1			1	short	EK	1	
Brentidae	Coleoptera	Brentidae	NULL	1		-1.15	0	0	0	1	iorder	0.5	iorder	1			1	semi-aquatic	iorder	0	
Caenidae	Ephemeroptera	Caenidae	NULL	1		-0.3	1	0	1	1-3	7, 45, QEK	0.5-1	7; 9:152	1	high-mod	46	0	short	8:3	1	
Calamoceratidae	Trichoptera	Calamoceratidae	NULL	1		-0.06	1	1	1	1-2	64	0.5	64	1	mod drift, far	in not as	QEK	1	1 months	EK, QEK	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
														adults						
Calocid/Helicophidae	Trichoptera	Calocid/Helicophidae	NULL	1		-0.06	1	1	1	1-2	19:14, QEK	2-3	19:14	1	some strong drifting taxa	44	1	1-2 months	EK	1
Calocidae	Diptera	Calocidae	NULL	1		-0.06	1	1	1	1-2	19:14, QEK	2-3	19:14	1	some strong drifting taxa	44	1	1-2 months	EK	1
Carabidae	Coleoptera	Carabidae	NULL	1		-1.15	0	0	0	1	QEK	unknown		1	mod		1	live at the edge of water	2:279	0
Ceinidae	Amphipoda	Ceinidae	NULL	1		0.16	1	0	0	many	72	0.25	72	0	low	62, QEK	1	fully aquatic	RS	1
Ceratopogonidae	Diptera	Ceratopogonidae	NULL	1		-0.35	1	0	0	1-4	70:467	0.2	73	0			1	short	EK	1
Chaoboridae	Diptera	Chaoboridae	NULL	1		-0.35	1	0	0	1	QEK	unknown		999			1	short	EK	1
Chironomidae	Diptera	Chironomidae	NULL	1		-0.39	0	0	0	1-4 or more	2:246, QEK	0.2	37:79	0	strong drifting	5; 43	0	few days to weeks	37:79	1
Chironominae	Diptera	Chironomidae	Chironominae	1		-0.39	0	0	0	1-4 or more	2:246, QEK	0.2	37:79	0	strong drifting	5; 43	0	few days to weeks	37:79	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
Chorismagriidae	Odonata	Chorismagriidae	NULL	1		-0.36	1	1	1	EK	1	EK	1	low		1	few weeks to 2-3 months (adults)	3:11 45; 71	1
Chrysomelidae	Coleoptera	Chrysomelidae	NULL	1		-1.15	0	0	0	70:419; 73	2	70:419	1			1	semi-aquatic	70:419	0
Cirolanidae	Isopoda	Cirolanidae	NULL	1		-0.56	0	0	0	QE	>1-3	?	0	limited	QE	1	fully aquatic	QE	1
Coenagrionidae	Odonata	Coenagrionidae	NULL	1		-0.24	1	1	1	EK	0.25-0.5	73; 74	1			1	few weeks to 2-3 months (adults)	3:11 45; 71	1
Coleoptera	Coleoptera	NULL	NULL	1		-1.15	0	0	0	order	0.5	order	1			1	semi-aquatic	order	0
Collembola	Collembola	NULL	NULL	1					0	QE	>1			limited	QWK	1	semi-aquatic	QE	0
Conoesucidae	Trichoptera	Conoesucidae	NULL	1		-0.06	1	1	1	EK	1-2	EK	1			1	1-2 months	EK	1
Corallanidae	Isopoda	Corallanidae	NULL	1		-0.56	0	0	0	QE	>1-3	?	0	limited	QE	1	fully aquatic	QE	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorgh	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
Corbiculidae	Veneroida	Corbiculidae	NULL	1		-2.09	0	0	0	1-2	48, QEK	0.2	48	0	low	3:12 43	1	fully aquatic	RS	1
Cordulephidae	Odonata	Cordulephidae	NULL	1		-0.96	0	0	0	1	15	0.75	73	1			1	few weeks to 2-3 months(adults)	3:11 45; 71	1
Corduliidae	Odonata	Corduliidae	NULL	1		-0.96	0	0	0	0.5	57	1.5	57	1	do not fly far away from the streams where they bred	3:11 52	1	few weeks to 2-3 months(adults)	3:11 45; 71	1
Corixidae	Hemiptera	Corixidae	NULL	1		-0.29	1	0	0	2-3	39:34	0.25	39:34	0	strong flyers	2:22 0	0	fully aquatic	2:21 3	1
Corophiidae	Amphipoda	Corophiidae	NULL	1		0.16	1	0	0	many	order	0.25	order	0	high (birds?)	62	0	fully aquatic	RS	1
Corydalidae	Megoptera	Corydalidae	NULL	1		-0.06	1	1	1	1	42:336	1-5	42:336	1			1	eggs 1-4 weeks and pupation on land. Adults short-lived	42:3 36	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
Culicidae	Diptera	Culicidae	NULL	1		-0.29	1	0	0	many	70:43, QEK	0.2	70:43	0		1	less than 4 weeks	73	1
Curculionidae	Coleoptera	Curculionidae	NULL	1		-1.15	0	0	0	1-2	QEK	0.25-0.5	70:422	0		1	live at the edge of water	2:287	0
Diphlebiidae	Odonata	Diphlebiidae	NULL	1		-0.36	1	1	1	1-2	73, QEK	1	73	1		1	few weeks to 2-3 months (adults)	3:1145; 71	1
Dipseudopsidae	Trichoptera	Dipseudopsidae	NULL	1		-0.06	1	1	1	1-2	QEK			1	mod	QEK	day-weeks	QEK	1
Diptera	Diptera	NULL	NULL	1		-0.35	1	1	0	1-many	ior order	0.5	ior order	0		1	short	ior order	1
Dixidae	Diptera	Dixidae	NULL	1		-0.35	1	1	0	>2	EK, QEK	0.5	EK	0		1	short	EK	1
Dolichopodidae	Diptera	Dolichopodidae	NULL	1		-0.35	1	0	0	>1	EK, QEK	0.5	EK	1		1	live at the edge of water	RM	0
Dugesidae	Seriata	Dugesidae	NULL	1		-0.43	0	0	0	Many	2:51	3.5	2:50	0	limited	QEK	fully aquatic	RS	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until repfoduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
Dytiscidae	Coleoptera	Dytiscidae	NULL	1		-0.81	0	0	0	1-2	31, QEK	0.5	27:4; 31: 1	1	strong flyers	32	0	semi-aquatic	2:28 2	0
Ecnomidae	Trichoptera	Ecnomidae	NULL	1		-0.06	1	1	1	1-2	52	0.33-1	9:152	1			1	1-2 months	EK	1
Elmidae	Coleoptera	Elmidae	NULL	1		-1.15	0	0	0	0.5-1	70:410, QEK	2-3	70:410	1	strong drifting	5; 44	0	short	29: 1	1
Empididae	Diptera	Empididae	NULL	1		-0.35	1	1	1	1-2	73, QEK	0.5	EK	1			1	short	EK	1
Ephemerellidae	Ephemeroptera	Ephemerellidae	NULL	1		-0.29 501	1	1	1	1-2	QEK			1	similar to Baetidae	QEK	1			1
Ephemeroptera	Ephemeroptera	NULL	NULL	1		-0.3	1	1	1	1-2	iorder	0.5-2	iorder	1			1	short	iorder	1
Ephydriidae	Diptera	Ephydriidae	NULL	1		-0.35	1	0	0	>2	73	0.25	EK	0			1	live at the edge of water	EK	0
Epiproctophora	Odonata	NULL	NULL	1		-0.96	0	0	0	0.5-1	iorder	1	iorder	1			1	few weeks to 2-3 months(adults)	iorder	1
Erpobdellida	N/A	Erpobdellida	NULL	1		-	0	0	0	up to	73	0.4	73	0			1	fully	RS	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
e		e				0.41			12								aquatic			
Eusiridae	Amphipoda	Eusiridae	NULL	1		0.16	1	0	0	many	72	0.25	72	0	high (birds?)	62	0	fully aquatic	RS	1
Eustheniidae	Plecoptera	Eustheniidae	NULL	1		0.38	1	1	1	1	60	2-3	2:191; 9:152	1	low - bad flyers	2:191; 3:1171	1-4 week (in autumn)	2:191; 3:1181; 60	1	
Gastropoda	NULL	NULL	NULL	1		-0.64	0	0	0	1-4	iorder, QEK	0.3	iorder	0			1	fully aquatic	RS	1
Gelastocoridae	Hemiptera	Gelastocoridae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0			1	semi-aquatic	2:213	0
Gerridae	Hemiptera	Gerridae	NULL	1		-0.56	0	0	0	1-2	41, QEK	0.5	41	1	strong flyers, but wingless forms	3:1208, QEK	1	live on water surface	2:213	0
Glossiphoniidae	N/A	Glossiphoniidae	NULL	1		-0.6	0	0	0	up to 12 (might not be up to 12)	iorder, QEK	0.4	iorder	0	low	QEK	1	fully aquatic	RS	1
Glossosomat	Trichoptera	Glossosomat	NULL	1		-	1	1	1	1-2	52,	0.75	52	1	some strong	44	1	1-2	EK	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
idae	ra	dae				0.06				QEK				drifting taxa			months			
Gomphidae	Odonata	Gomphidae	NULL	1		-0.96	0	0	0	0.5	57	1.5	57	1	do not fly far away from the streams where they bred	3:11 52	1	few weeks to 2-3 months(adults)	3:11 45; 71	1
Gomphomacromiidae	Odonata	Gomphomacromiidae	NULL	1		-0.96	0	0	0	0.5	57	1.5	57	1	do not fly far away from the streams where they bred	3:11 52	1	few weeks to 2-3 months(adults)	3:11 45; 71	1
Grapsidae	Decapoda	Grapsidae	NULL	1		-0.07765	1	1	1	1			1			1	fully aquatic		1	
Gripopterygidae	Plecoptera	Gripopterygidae	NULL	1		0.38	1	1	1	1	7	0.5-2	EK	1	low - bad flyers	2:19 1; 3:11 71	1	1-4 week	2:19 1; 3:11 81	1
Gyrinidae	Coleoptera	Gyrinidae	NULL	1		-1.15	0	0	0	1	EK	0.5	EK	1	strong	QEK	0	fully aquatic	2:28 0	1
Haliplidae	Coleoptera	Haliplidae	NULL	1		-	0	0	0	1	73	0.5	73	1			1	fully	2:28	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
	a					1.83											aquatic	0	
Hebridae	Hemiptera	Hebridae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0		1	live on water surface	2:213	0
Helicopsychidae	Trichoptera	Helicopsychidae	NULL	1		-0.06	1	1	1	2	EK	0.5	EK	1	some strong drifting taxa	5; 63	1-2 months	EK	1
Hemicordulidae	Odonata	Hemicordulidae	NULL	1		-0.96	0	0	0	1	73	0.5	3:1152	1	strong flyers	3:1154	few weeks to 2-3 months (adults)	3:1145; 71	1
Heteroceridae	Coleoptera	Heteroceridae	NULL	1		-1.15	0	0	0	1-2	QEK	unknown		1	mod	QEK	live at the edge of water	2:288	0
Hirudinea	N/A	NULL	NULL	1		-0.6	0	0	0	up to 12	order	0.4	order	0		1	fully aquatic	RS	1
Hydraenidae	Coleoptera	Hydraenidae	NULL	1		-1.15	0	0	0	1-2	70:398, QEK	0.75	70:398	1		1	varies strongly within life-stages and	2:285	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorghum	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
																	genera		
Hydrobiidae	Neotaenioglossa	Hydrobiidae	NULL	1		-1.82	0	0	0	3	47	0.3	order	0		1	fully aquatic	RS	1
Hydrobiosidae	Trichoptera	Hydrobiosidae	NULL	1		-0.06	1	1	1	1		1	EK	1	some strong drifting taxa	44	1-2 months	EK	1
Hydrochidae	Coleoptera	Hydrochidae	NULL	1		-1.15	0	0	0	1-2	EK, QEK	0.5	EK	1		1	unknown		999
Hydrometridae	Hemiptera	Hydrometridae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0		1	live on water surface	2:213	0
Hydrophilidae	Coleoptera	Hydrophilidae	NULL	1		-0.89	0	0	0	1-2?	73, QEK	0.25	70: 392	1		1	semi-aquatic	70: 361	0
Hydropsychidae	Trichoptera	Hydropsychidae	NULL	1		-0.5	0	0	0	1-2	7, QEK	0.75	7	1	mod-high	46; 63, QEK	1-2 months	EK	1
Hydroptilidae	Trichoptera	Hydroptilidae	NULL	1		-0.06	1	0	0	1-many	EK, QEK	0.25-1	EK	0	some strong drifting taxa	46; 63	few weeks	EK	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
Hygrobiidae	Coleoptera	Hygrobiidae	NULL	1		-1.15	0	0	0	1-2	QEK	unknown	1	mod	QEK	1	fully aquatic	2:280	1	
Hypolestidae	Odonata	Hypolestidae	NULL	1		-0.36	1	1	1				1			1	weeks	QEK	1	
Hyriidae	Unionoida	Hyriidae	NULL	1		-2.09	0	0	0	2	4:63	3.5	4:63	1	low	3:1243	1	fully aquatic	RS	1
Isostictidae	Odonata	Isostictidae	NULL	1		-0.36	1	1	1	1	EK	1	EK	1	strong	QEK	0	few weeks to 2-3 months(adults)	3:1145; 71	1
Janiridae	Isopoda	Janiridae	NULL	1		-0.56	0	0	0	1-2	53	0.5	53	1			1	fully aquatic	RS	1
Lepidoptera	Lepidoptera	NULL	NULL	1		-0.06	1	1	1	1-3	73	0.25-0.75	73	1			1	1-4 week	73	1
Leptoceridae	Trichoptera	Leptoceridae	NULL	1		-0.06	1	1	1	0.5-2	7, 51	0.5-2	7, 51	1	some strong drifting taxa	6	1	1-2 months	EK	1
Leptophlebiidae	Ephemeroptera	Leptophlebiidae	NULL	1		-0.3	1	1	1	1-2	7, 45	0.5-1	7; 9:152	1	some strong drifting taxa (Atalophle	5; 44; 63, QEK	1	short (November to March)	7	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-inv	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until repfoduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
														bioides), mod						
Leptopodidae	Hemiptera	Leptopodidae	NULL	1		-0.56	0	0	0	1-2	QEK		1	mod	QEK	1	semi-aquatic	QEK	0	
Lestidae	Odonata	Lestidae	NULL	1		-0.68	0	0	0	1	73	0.5	3:1152	1	do not fly far away from the streams where they bred	3:1152	1	adults live up to 9 months	3:1145; 71; 73	0
Libellulidae	Odonata	Libellulidae	NULL	1		-1.53	0	0	0	1	15	1	57	1	strong flyers	3:1154	0	few weeks to 2-3 months(adults)	3:1145; 71	1
Limnichidae	Coleoptera	Limnichidae	NULL	1		-1.15	0	0	0	1-2	QEK			1	mode	QEK	1	fully aquatic	QEK	1
Lindeniidae	Odonata	Lindeniidae	NULL	1		-0.96	0	0	0	0.5-1	57, QEK	1.5	57	1	do not fly far away from the streams where they bred	3:1152	1	few weeks to 2-3 months(adults)	3:1145; 71	1
Lymnaeidae	Basomma	Lymnaeidae	NULL	1		-	0	0	0	1-2	73	0.3	73	1			1	fully	RS	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
	tophora					0.63											aquatic			
Macromiidae	Odonata	Macromiidae	NULL	1		-0.96	0	0	0	0.5-1	57, QEK	1.5	57	1	do not fly far away from the streams where they bred	3:11 52	1	few weeks to 2-3 months(adults)	3:11 45; 71	1
Megapodagrionidae	Odonata	Megapodagrionidae	NULL	1		-0.36	1	0	0	1	73	1	73	1		1	adults live up to 7 months	3:11 45; 71; 73	0	
Melitidae	Amphipoda	Melitidae	NULL	1		0.16	1	1	1	1-2	QEK	>0.5	QEK	1	low-mod	QEK	1	fully aquatic	QEK	1
Mesoveliidae	Hemiptera	Mesoveliidae	NULL	1		-0.56	0	0	0	3	39:72	0.25	39:34	0		1	live on water surface	2:21 3	0	
Microsporidiae	Coleoptera	Microsporidiae	NULL	1		-1.15	0	0	0	perhaps many	QEK			0	low	1	1	fully aquatic	QEK	1
Muscidae	Diptera	Muscidae	NULL	1		-0.35	1	0	0	many	QEK	unknown		0		1	1	semi-aquatic	80:8 57	0
Naucoridae	Hemiptera	Naucoridae	NULL	1		-0.56	0	0	0	1	40	0.5	40	1		1	1	fully aquatic	2:21 3	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no	Time until reproduction (a)	Ref time until repoduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
Nematomorpha	NULL	NULL	NULL	1		-1.1	0	0	0	not many	QEK	unknown	1			1	few months in water, then in host (partly terrestrial)	EK	0	
Nemertea	NULL	NULL	NULL	1				0	unknown					low	1	1	fully aquatic	QEK	1	
Nepidae	Hemiptera	Nepidae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0		1	fully aquatic	2:213	1	
Neurorthidae	Neuroptera	Neurorthidae	NULL	1		-0.06	1	1	1	1	order	0.5	73	1	low-mod	1	Pupation and adult on land	2:229	1	
Noteridae	Coleoptera	Noteridae	NULL	1		-1.15	0	0	0	1	80:314	0.5	80:314	1		1	fully aquatic	2:282	1	
Notonectidae	Hemiptera	Notonectidae	NULL	1		-0.86	0	0	0	2-3	39:34	0.25	39:34	0	strong flyers	3:1208	0	fully aquatic	2:213	1
Nymphulinae (Group A)	Lepidoptera	Pyralidae	Nymphulinae (Group A)	1		-0.06	1	1	1	1-3	73	0.25-0.75	73	1		1	1-4 week	73	1	

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
Ochteridae	Hemiptera	Ochteridae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0		1	semi-aquatic	2:213	0
Odontoceridae	Trichoptera	Odontoceridae	NULL	1		-0.06	1	1	1	1	23:2	0.75	23:2	1		1	1-2 months	EK	1
Oligochaeta	NULL	NULL	NULL	1		-1.1	0	0	0	1-many	EK	0.25-1	EK	0	some strong drifting taxa	63	fully aquatic	RS	1
Oniscidae	Isopoda	Oniscidae	NULL	1		-0.56	0	0	0	1-2	53	0.5	53	1		1	semi-aquatic	53	0
Ornithobdellidae	N/A	Ornithobdellidae	NULL	1		-0.6	0	0	0	up to 12	order	0.4	order	0	bird leaches	QEK	fully aquatic	RS	1
Orthocladinae	Diptera	Chironomidae	Orthocladinae	1		-0.39	0	0	0	1-4	2:246	0.2	37:79	0	strong drifting	5; 43	few days to weeks	37:79	1
Osmylidae	Neuroptera	Osmylidae	NULL	1		-0.06	1	1	1	1	73	0.5	73	1		1	Pupation and adult on land	2:229	1
Palaemonidae	Decapoda	Palaemonidae	NULL	1		0.26	1	1	1	1-2	73, QEK	1-2	73	1	mod	QEK	fully aquatic	RS	1
Parastacidae	Decapoda	Parastacidae	NULL	1		-0.08	1	1	1	<=1	1:89, QEK	1-5	1: 22; 55	1	low	QEK	fully aquatic	RS	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
Parastacidae	Decapoda	Parastacidae	NULL	1		-0.08	1	1	1	1:89	1-5	1: 22; 55	1			1	fully aquatic	RS	1
Philopotamidae	Trichoptera	Philopotamidae	NULL	1		-0.06	1	1	1	49	0.75	49	1			1	1-2 months	EK	1
Philorheithridae	Trichoptera	Philorheithridae	NULL	1		-0.06	1	1	1	EK	1	EK	1			1	1-2 months	EK	1
Physidae	Basommatophora	Physidae	NULL	1		-1.67	0	0	0	73	0.3	73	1			1	fully aquatic	RS	1
Planorbidae	Basommatophora	Planorbidae	NULL	1		-1.94	0	0	0	73	0.3	73	1			1	fully aquatic	RS	1
Plecoptera	Plecoptera	NULL	NULL	1		0.38	1	1	1	1	2-3	1	1	low - bad flyers	1	1	1-4 week	1	1
Pleidae	Hemiptera	Pleidae	NULL	1		-0.56	0	0	0	39:34	0.25	39:34	0			1	fully aquatic	2:213	1
Polycentropodidae	Trichoptera	Polycentropodidae	NULL	1		-0.06	1	1	1	EK	0.5-1	EK	1			1	1-2 months	EK	1
Polychaeta	NULL	NULL	NULL	1		-1.1	0	0	0				1			1			1
Prosopistomatidae	Ephemeroptera	Prosopistomatidae	NULL	1		-0.3	1	1	1	QEK			1	mod	QEK	1	weeks to < 2 months	QW2K	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
Protoneuridae	Odonata	Protoneuridae	NULL	1		-0.36	1	1	1	EK	1	EK	1	do not fly far away from the streams where they bred	3:1152	1	few weeks to 2-3 months (adults)	3:1145; 71	1
Psephenidae	Coleoptera	Psephenidae	NULL	1		-1.15	0	0	0	2:290	2	2:290	1	strong (from genetics)	QEK	0	4 weeks (adults)	2:290	1
Pseudocorduliidae	Odonata	Pseudocorduliidae	NULL	1		-0.96	0	0	0	57, QEK	1.5	57	1	do not fly far away from the streams where they bred	3:1152	1	few weeks to 2-3 months (adults)	3:1145; 71	1
Psychodidae	Diptera	Psychodidae	NULL	1		-0.35	1	0	0	EK	0.25	EK	0			1	live at the edge of water	2:245	0
Ptilodactylidae	Coleoptera	Ptilodactylidae	NULL	1		-1.15	0	0	0	70:405	2-3	70:405	1			1	live at the edge of water	2:291	0
Pyralidae	Lepidoptera	Pyralidae	NULL	1		-0.06	1	1	1	73	0.25-0.75	73	1			1	1-4 week	73	1
Richardsonia	N/A	Richardsonia	NULL	1		-0.6	0	0	0	up to 73	0.4	73	0			1	fully	RS	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorghum	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
nidae		nidae							12								aquatic		
Saldidae	Hemiptera	Saldidae	NULL	1	-0.56	0	0	0	2-3	39:34	0.25	39:34	0			1	semi-aquatic	2:213	0
Sciomyzidae	Diptera	Sciomyzidae	NULL	1	-0.35	1	0	0	1-4	QEK	unknown		0	mod	QEK	1	short	EK	1
Scirtidae	Coleoptera	Scirtidae	NULL	1	-1.15	0	0	0	1	45	0.75	45, 70:402	1			1	short	70:402	1
Sialidae	Megeloptera	Sialidae	NULL	1	-0.06	1	1	1	1	42:336	1-5	42:336	1			1	eggs 1-4 weeks and pupation on land. Adults short-lived	42:336	1
Simuliidae	Diptera	Simuliidae	NULL	1	-0.35	1	0	0	>=2	70:534, QEK	0.25-0.5	70:534	0	poor dispersers (genetics)	5; 63, QEK	1	short	2:238	1
Sisyridae	Neuroptera	Sisyridae	NULL	1	-0.06	1	1	1	1	order	0.5	73	1	mod	QEK	1	Pupation and adult on land	2:229	1
Spercheidae	Coleoptera	Spercheidae	NULL	1	-	0	0	0	1	73	0.25	70:392	1			1	semi-	70:	0

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until reproduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment
	a					0.89											aquatic	361	
Sphaeriidae	Veneroida	Sphaeriidae	NULL	1		-2.09	0	0	0	>2	EK	0.2	EK	0	low	3:1243	1	fully aquatic	RS 1
Sphaeromatidae	Isopoda	Sphaeromatidae	NULL	1		-0.56	0	0	0	1-2	53	0.5	53	1	low	QEK	1	fully aquatic	RS 1
Staphlinidae	Coleoptera	Staphylinidae	NULL	1		-1.15	0	0	0	1-2	QEK	unknown		1			live at the edge of water	2:284	0
Stratiomyidae	Diptera	Stratiomyidae	NULL	1		-0.35	1	1	1	>2	70:467, QEK	0.5	73	1			1	few days	73 1
Sundathelphusidae	Decapoda	Sundathelphusidae	NULL	1		-0.08	1	1	1	1	QEK	1	QEK	1	low	QEK	1	fully aquatic	QEK 1
Synlestidae	Odonata	Synlestidae	NULL	1		-0.36	1	1	1	1	EK	1	EK	1			1	few weeks to 2-3 months (adults)	3:1145; 71 1
Synthemistidae	Odonata	Synthemistidae	NULL	1		-0.96	0	0	0	1	69	1	69	1	do not fly far away from the streams where they bred	3:1152	1	few weeks to 2-3 months (adults)	3:1145; 71 1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-invertebrate	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until repoduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
Syrphidae	Diptera	Syrphidae	NULL	1		-0.35	1	0	0	0.25			0			1			1	
Tabanidae	Diptera	Tabanidae	NULL	1		-0.35	1	0	0	>1	73, QEK	0.75-1.5	73	1		1	semi-aquatic + pupation on land	37:80; 38:7	0	
Talitridae	Amphipoda	Talitridae	NULL	1		0.16	1	0	0	many	iorder	0.25	iorder	0	high (birds?)	62	0	fully aquatic	RM	0
Tanyderidae	Diptera	Tanyderidae	NULL	1		-0.35	1	1	1	1-2	70:467	1	EK	1		1	short	EK	1	
Tanypodinae	Diptera	Chironomidae	Tanypodinae	1		-0.39	0	0	0	1-4	2:246	0.2	37:79	0	strong drifting	5; 43	0	few days to weeks	37:79	1
Tasimiidae	Trichoptera	Tasimiidae	NULL	1		-0.06	1	1	1	2	EK	0.5	EK	1		1	1-2 months	EK	1	
Telephlebiidae	Odonata	Telephlebiidae	NULL	1		-0.96	0	0	0	0.5-1	EK	1-2	EK	1		1	semi-aquatic	71:11	0	
Temnocephalidae	Temnocephalida	Temnocephalidae	NULL	1		-0.43	0	0	0	Many	iorder	<0.1	iorder	0		1	fully aquatic	RS	1	
Thaumaleidae	Diptera	Thaumaleidae	NULL	1		-0.35	1	1	1	1	EK	0.75-1	EK	1	low	QEK	1	short	EK	1

Lowest ID in Qld database	Order	Family	Sub-family	Macro-inv	ID too coarse	Sorg	S assessment	SPEAR assessment nQLD	Number of generations per year	Ref no generations	Time until reproduction (a)	Ref time until repoduction	G assessment	Dispersal capacity	Ref dispersal	Dispersal assessment	Duration of life stages out of water	Ref out of water	out of water assessment	
Thiaridae	Neotaenioglossa	Thiaridae	NULL	1		-1.23	0	0	0	3	iorder	0.3	iorder	0	presumably low		1	fully aquatic	RS	1
Tipulidae	Diptera	Tipulidae	NULL	1		-0.35	1	1	1	0.5-2	73	0.25-1	2:236	1	mod	QEK	1	short	73	1
Trichoptera	Trichoptera	NULL	NULL	1		-0.06	1	1	1	1	iorder	0.5	iorder	1			1	1-2 months	iorder	1
Veliidae	Hemiptera	Veliidae	NULL	1		-0.56	0	0	0	2-3	39:34	0.25	39:34	0			1	live on water surface	2:213	0
Viviparidae	Architaenioglossa	Viviparidae	NULL	1		-1.50151	0	0	0	1				1	poor dispersers (genetics)	QEK	1	fully aqitic	QEK	1
Zygoptera	Odonata	NULL	NULL	1		-0.96	0	0	0	0.5-1	iorder	1	iorder	1			1	few weeks to 2-3 months(adults)	iorder	1

Appendix 3. References for traits in appendix 2.

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EK	Expert knowledge - Workshop in EPA Victoria in March 2009 in Melbourne. Participants: Leon Metzeling (EPA), Richard Marchant (Museum Victoria), Ros St.Clair (EPA Victoria), Ralf Schäfer (now University Koblenz-Landau), Ben Kefford (now University of Technology Sydney)
iorder	Interpolated from Order
RS	Ralf Schäfer (now University Koblenz-Landau), personal communication

	(February 2009)
RM	Richard Marchant (Museum Victoria), personal communication (February 2009)
QEK	Queensland specific Expert knowledge - Workshop at DSITIA in June 2012 in Brisbane. Participants: Satish Choy, Jason Dunlop & Raj Prasad (DSITIA), Jane Hughes (Griffith University), Richard Pearson (James Cook University), Ben Kefford (University of Technology Sydney), input also from Jon Marshall and Alisha Steward (DSITIA)

Appendix 4. Preliminary classification of stream diatoms collected in the sampling program as sensitive or tolerant of herbicides.

SPEAR taxa are designated as sensitive to herbicides (i.e. a SPEcies At Risk) if SPEAR taxa=1, else not sensitive to herbicides if SPEAR taxa = 0 with-999 indicates that a taxa is unknown.

Species	Genus	Order	SPEARgenus	Explanation
<i>Achnanthes exigua</i>	<i>Achnanthes</i>	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
<i>Achnanthes impexa</i>	<i>Achnanthes</i>	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
<i>Achnanthes nodosa</i>	<i>Achnanthes</i>	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
<i>Achnanthes oblongella</i>	<i>Achnanthes</i>	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
<i>Achnantheidium minutissimum</i>	<i>Achnantheidium</i>	Achnanthes	1	Genus extrapolation
<i>Achnantheidium minutissimum</i> var. <i>affine</i>	<i>Achnantheidium</i>	Achnanthes	1	Genus extrapolation
<i>Achnantheidium</i> sp.	<i>Achnantheidium</i>	Achnanthes	1	Bluewater - Response to 500ug/L 2,4-D (sig dose response p-value = <0.001) and Diuron (p-value = 0.008), no sig diff other herbs.
<i>Actinocyclus normanii</i>	<i>Actinocyclus</i>	Coscinodiscales	-9999	excluded
<i>Amphora coffeaeformis</i>	<i>Amphora</i>	Thalassiosiphysales	1	Genus extrapolation
<i>Amphora coffeaeformis</i> var. <i>borealis</i>	<i>Amphora</i>	Thalassiosiphysales	1	Genus extrapolation
<i>Amphora delicatissima</i>	<i>Amphora</i>	Thalassiosiphysales	1	Genus extrapolation
<i>Amphora holsatica</i>	<i>Amphora</i>	Thalassiosiphysales	1	Genus extrapolation

Species	Genus	Order	SPEARgenus	Explanation
Amphora montana	Amphora	Thalassiosiphysales	1	Genus extrapolation
Amphora pediculus	Amphora	Thalassiosiphysales	1	Genus extrapolation
Amphora sp.	Amphora	Thalassiosiphysales	1	Bluewater Ck – significant dose reponse
Amphora thumensis	Amphora	Thalassiosiphysales	1	Genus extrapolation
Aulacoseira ambigua	Aulacoseira	Aulacoseirales	-9999	excluded
Aulacoseira crenulata	Aulacoseira	Aulacoseirales	-9999	excluded
Aulacoseira granulata	Aulacoseira	Aulacoseirales	-9999	excluded
Bacillaria paxillifer	Bacillaria	Bacillariales	-9999	Order extrapolation - Bacillariales tolerant
Brachysira brachysira	Brachysira	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Brachysira styriaca	Brachysira	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Caloneis aerophila	Caloneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Caloneis silicula	Caloneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Cocconeis placentula	Cocconeis	Achnanthes	0	Barratta- no significant response. Gowrie Creek - significant decline in low light only, very low control levels of healthy cells and high variability.
Cocconeis placentula var. linearis	Cocconeis	Achnanthes	0	Genus extrapolation
Cocconeis pseudothumensis	Cocconeis	Achnanthes	0	Genus extrapolation
Craticula accomoda	Craticula	Naviculales	-9999	Order extrapolation - Naviculales tolerant

Species	Genus	Order	SPEARgenus	Explanation
Craticula cuspidata	Craticula	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Craticula halophila	Craticula	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Craticula halophiloides	Craticula	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Craticula molestiformis	Craticula	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Craticula riparia	Craticula	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Cyclostephanos tholiformis	Cyclostephanos	Thalassiosirales	-9999	excluded
Cyclotella comensis	Cyclotella	Thalassiosirales	-9999	excluded
Cyclotella meneghiniana	Cyclotella	Thalassiosirales	-9999	excluded
Cyclotella pseudostelligera	Cyclotella	Thalassiosirales	-9999	excluded
Cyclotella stelligera	Cyclotella	Thalassiosirales	-9999	excluded
Cymbella affinis	Cymbella	Cymbellales	1	Genus extrapolation
Cymbella cistula	Cymbella	Cymbellales	1	Genus extrapolation
Cymbella delicatula	Cymbella	Cymbellales	1	Genus extrapolation
Cymbella laevis	Cymbella	Cymbellales	1	Genus extrapolation
Cymbella lanceolata	Cymbella	Cymbellales	1	Bluewater - Sig. dose response Atrazine (p-value = 0.004). Liverpool ck & Aligator Ck - Non sig dose response. Barratta creek high light >10%healthy cells at 50ug/L all dead at 500ug/L
Cymbella tumida	Cymbella	Cymbellales	1	Genus extrapolation
Diadesmis confervacea	Diadesmis	Naviculales	-9999	Order extrapolation - Naviculales tolerant

Species	Genus	Order	SPEARgenus	Explanation
Diploneis elliptica	Diploneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Diploneis ovalis	Diploneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Diploneis parma	Diploneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Diploneis smithii	Diploneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Diploneis subovalis	Diploneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Encyonema gracilis	Encyonema	Cymbellales	1	Genus extrapolation
Encyonema mesiana	Encyonema	Cymbellales	1	Genus extrapolation
Encyonema minuta	Encyonema	Cymbellales	1	Liverpool Ck - Sig response in low light at highest concentration level only.
Encyonema silesiacum	Encyonema	Cymbellales	1	Genus extrapolation
Encyonopsis cesatii	Encyonopsis	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Encyonopsis microcephala	Encyonopsis	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Encyonopsis perborealis	Encyonopsis	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Entomoneis alata	Entomoneis	Surirellales	-9999	No data
Entomoneis costata	Entomoneis	Surirellales	-9999	No data
Eolimna minima	Eolimna	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Eolimna subminuscula	Eolimna	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Epithemia adnata	Epithemia	Rhopalodiales	0	Genus extrapolation
Epithemia cv. turgida	Epithemia	Rhopalodiales	0	Alligator Ck - No response - very low numbers of healthy cells in controls.

Species	Genus	Order	SPEARgenus	Explanation
Epithemia sorex	Epithemia	Rhopalodiales	0	Genus extrapolation
Eunotia arcus	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia bilunaris v. mucophila	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia exigua	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia fallax	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia implicata	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia incisa	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia minor	Eunotia	Eunotiales	0	Bluewater Ck/ Alligator Ck/ Gowrie Ck - No response - very low numbers of healthy cells in controls and high variability between treatments.
Eunotia muscicola var tridentula	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia paludosa	Eunotia	Eunotiales	0	Genus extrapolation
Eunotia pectinalis	Eunotia	Eunotiales	0	Genus extrapolation
Fallacia pygmaea	Fallacia	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Fallacia tenera	Fallacia	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Fragilaria capucina var capucina	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria capucina var gracilis	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive

Species	Genus	Order	SPEARgenus	Explanation
Fragilaria capucina var perminuta	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria capucina var rumpens	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria capucina var vaucheriae	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria crotonensis	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria leptostriata	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria sp.	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilaria tenera	Fragilaria	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Fragilariforma virescens	Fragilariforma	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Frustulia rhomboides	Frustulia	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Frustulia vulgaris	Frustulia	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Geissleria decussis	Geissleria	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Gomphonema affine	Gomphonema	Cymbellales	1	Bluewater Ck (<i>cf. lanceolatum</i>) Sig. dose response to all 8 herbicides
Gomphonema angustum	Gomphonema	Cymbellales	1	Liverpool Ck (<i>cf. angustatum</i>) non sig dose response high and low light. Bluewater Ck - non sig response.
Gomphonema angustum var "subminutum"	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema bohemicum	Gomphonema	Cymbellales	1	Genus extrapolation

Species	Genus	Order	SPEARgenus	Explanation
Gomphonema clevei	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema gracile	Gomphonema	Cymbellales	1	Alligator – Non sig dose response low light
Gomphonema micropus	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema minutum	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema olivaceum	Gomphonema	Cymbellales	1	Aligator Ck - Dose response
Gomphonema parvulum	Gomphonema	Cymbellales	1	Barratta – no sig response – highly variable b/w treatments. Gowrie - dose reponse, sig at high light only.
Gomphonema parvulum var exillissimum	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema pseudoaugar	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema pseudotenellum	Gomphonema	Cymbellales	1	Genus extrapolation
Gomphonema truncatum	Gomphonema	Cymbellales	1	Alligator Ck - Low light dose response
Gyrosigma attenuatum	Gyrosigma	Naviculales	0	Genus extrapolation
Gyrosigma nodiferum	Gyrosigma	Naviculales	0	Genus extrapolation
Gyrosigma parkerii	Gyrosigma	Naviculales	0	Genus extrapolation
Gyrosigma spencerii	Gyrosigma	Naviculales	0	Barratta Ck - No response
Haslea spicula	Haslea	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Hippodonta capitata	Hippodonta	Naviculales	-9999	Order extrapolation - Achnanthes sensitive

Species	Genus	Order	SPEARgenus	Explanation
Karayevia laterostrata	Karayevia	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Kolbesia kolbei	Kolbesia	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Kolbesia ploenensis	Kolbesia	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Luticola goeppertiana	Luticola	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Luticola mutica	Luticola	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Mastogloia elliptica	Mastogloia	Mastogloiales	-9999	
Melosira varians	Melosira	Melosirales	0	Barratta Ck - Slight reponse at low light, high % of healthy cells at 500ug/L.
Meridion circulare	Meridion	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Navicella pusilla	Navicella	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Navicula cari	Navicula	Naviculales	0	Genus extrapolation
Navicula cincta	Navicula	Naviculales	0	Genus extrapolation
Navicula constans v. symmetrica	Navicula	Naviculales	0	Genus extrapolation
Navicula cryptocephala	Navicula	Naviculales	0	Barratta Ck / Alligator Ck - No response
Navicula cryptotenella	Navicula	Naviculales	0	Genus extrapolation
Navicula difficillima	Navicula	Naviculales	0	Genus extrapolation
Navicula duerrenbergiana	Navicula	Naviculales	0	Genus extrapolation
Navicula gottlandica	Navicula	Naviculales	0	Genus extrapolation
Navicula gregaria	Navicula	Naviculales	0	Barratta Ck/ Liverpool Ck - No diff bw controls & treatments in rapid tests

Species	Genus	Order	SPEARgenus	Explanation
Navicula heimansoides	Navicula	Naviculales	0	Genus extrapolation
Navicula kotschyii	Navicula	Naviculales	0	Genus extrapolation
Navicula lanceolata	Navicula	Naviculales	0	Genus extrapolation
Navicula leptostriata	Navicula	Naviculales	0	Genus extrapolation
Navicula libonensis	Navicula	Naviculales	0	Genus extrapolation
Navicula menisculoides	Navicula	Naviculales	0	Genus extrapolation
Navicula menisculus	Navicula	Naviculales	0	Genus extrapolation
Navicula muraliformis	Navicula	Naviculales	0	Genus extrapolation
Navicula notha	Navicula	Naviculales	0	Genus extrapolation
Navicula peregrina	Navicula	Naviculales	0	Genus extrapolation
Navicula phyllepta	Navicula	Naviculales	0	Genus extrapolation
Navicula pseudokotschyii	Navicula	Naviculales	0	Genus extrapolation
Navicula radiosa	Navicula	Naviculales	0	Alligator Ck/ Liverpool Ck - No diff bw controls & treatments at any concentration or light level.
Navicula recens	Navicula	Naviculales	0	Genus extrapolation
Navicula rhynchocephala	Navicula	Naviculales	0	Genus extrapolation
Navicula schroeterii	Navicula	Naviculales	0	Barratta Ck - >65% healthy at 500ug/L, no response high or low light.
Navicula soehrensii var muscicola	Navicula	Naviculales	0	Genus extrapolation

Species	Genus	Order	SPEARgenus	Explanation
Navicula splendicula	Navicula	Naviculales	0	Genus extrapolation
Navicula subminiscula	Navicula	Naviculales	0	Genus extrapolation
Navicula submuralis	Navicula	Naviculales	0	Genus extrapolation
Navicula subrhyncephala	Navicula	Naviculales	0	Genus extrapolation
Navicula tenelloides	Navicula	Naviculales	0	Genus extrapolation
Navicula tripunctata	Navicula	Naviculales	0	Genus extrapolation
Navicula trivialis	Navicula	Naviculales	0	Genus extrapolation
Navicula vandamii	Navicula	Naviculales	0	Genus extrapolation
Navicula veneta	Navicula	Naviculales	0	Liverpool Ck - No response at either light level or concentration level.
Navicula viridula	Navicula	Naviculales	0	Genus extrapolation
Navicula viridula v. germanii	Navicula	Naviculales	0	Genus extrapolation
Navicula viridula v. rostellata	Navicula	Naviculales	0	Genus extrapolation
Nitzschia accula	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia acicularis	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia aff. Tropica	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia agnita	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia amphibia	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia angustatula	Nitzschia	Bacillariales	0	Genus extrapolation

Species	Genus	Order	SPEARgenus	Explanation
Nitzschia angustiforaminata	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia braunii	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia capitellata	Nitzschia	Bacillariales	0	Gowrie- non sig drop at highest conc high light.
Nitzschia dissipata	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia diversa	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia dubia	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia elegantula	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia filiformis	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia fonticola	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia fossilis	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia frustulum	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia gessneri	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia graciliformis	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia gracilis	Nitzschia	Bacillariales	0	Liverpool Ck - No diff bw controls & treatments in rapid tests
Nitzschia inconspicua	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia lacuum	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia liebetruthii	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia linearis subtilis	Nitzschia	Bacillariales	0	Genus extrapolation

Species	Genus	Order	SPEARgenus	Explanation
Nitzschia microcephala	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia obtusa	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia palea	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia palea thin variety	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia paleaceae	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia paleaformis	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia perminuta	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia pumilla	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia pura	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia recta	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia reversa	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia sigmoideae	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia sinuata var. tabellaria	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia sociabilis	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia solita	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia subacicularis	Nitzschia	Bacillariales	0	Genus extrapolation

Species	Genus	Order	SPEARgenus	Explanation
Nitzschia subcapitata	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia valdecostata	Nitzschia	Bacillariales	0	Genus extrapolation
Nitzschia vermicularis	Nitzschia	Bacillariales	0	Genus extrapolation
Opephora olsenii	Opephora	Fragilariales	-9999	Order extrapolation - Naviculales tolerant
Pinnularia appendiculata	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia borealis	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia braunii	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia gibba	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia legumen	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia microstauron	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia similis	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia spp.	Pinnularia	Naviculales	0	Liverpool Ck/ Barratta Ck - No diff bw controls & treatments in rapid tests
Pinnularia subcapitata	Pinnularia	Naviculales	0	Genus extrapolation
Pinnularia viridula	Pinnularia	Naviculales	0	Genus extrapolation
Placoneis clementis	Navicula	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Placoneis elginensis	Placoneis	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Planothidium delicatulum	Planothidium	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Planothidium frequentissimum	Planothidium	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive

Species	Genus	Order	SPEARgenus	Explanation
Planothidium granum	Planothidium	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Planothidium lanceolatum	Planothidium	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Pleurosigma attenuatum	Pleurosigma	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Pleurosigma elongatum	Pleurosigma	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Psammothidium bioretii	Psammothidium	Achnanthes	-9999	Order extrapolation - Achnanthes tolerant
Psammothidium saccula	Psammothidium	Achnanthes	-9999	Order extrapolation - Achnanthes tolerant
Pseudostaurosira brevistriata	Pseudostaurosira	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Reimeria sinuata	Reimeria	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Rhoicosphenia abbreviata	Rhoicosphenia	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive
Rhopalodia brebissonii	Rhopalodia	Rhopalodiales	1	Genus extrapolation
Rhopalodia gibba	Rhopalodia	Rhopalodiales	1	Genus extrapolation
Rhopalodia musculus	Rhopalodia	Rhopalodiales	1	Alligator Ck - Dose response at high and low light levels.
Rossithidium linearis	Rossithidium	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Rossithidium pusilla	Rossithidium	Achnanthes	-9999	Order extrapolation - Achnanthes sensitive
Sellaphora pupula	Sellaphora	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Sellaphora seminulum	Sellaphora	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Stauroneis anceps	Stauroneis	Naviculales	-9999	Order extrapolation - Naviculales tolerant
Staurophora wislouchii	Staurophora	Cymbellales	-9999	Order extrapolation - Cymbellales sensitive

Species	Genus	Order	SPEARgenus	Explanation
Staurosirella pinnata	Staurosirella	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Stenopterobia curvula	Stenopterobia	Surirellales	-9999	excluded
Surirella biseriata	Surirella	Surirellales	-9999	excluded
Surirella brebissonii	Surirella	Surirellales	-9999	excluded
Surirella elegans	Surirella	Surirellales	-9999	excluded
Surirella linearis	Surirella	Surirellales	-9999	excluded
Surirella ovalis	Surirella	Surirellales	-9999	excluded
Surirella robusta	Surirella	Surirellales	-9999	excluded
Synedra acus	Synedra	Fragilariales	1	Alligator Ck - Dose response
Synedra ulna	Synedra	Fragilariales	1	Liverpool & Gowrie sig dose response at low light, non sig dose reponse at high light. Barratta non sig dose response at high light only. Alligator dose reponse to glyphosate. Bluewater Ck - treatments lower than controls in all 8 herbs. Sig dose response.
Tabularia fasciculata	Tabularia	Fragilariales	-9999	Order extrapolation - Fragilariales sensitive
Tryblionella apiculata	Tryblionella	Bacillariales	-9999	Order extrapolation - Bacillariales tolerant
Tryblionella calida	Tryblionella	Bacillariales	-9999	Order extrapolation - Bacillariales tolerant
Tryblionella debilis	Tryblionella	Bacillariales	-9999	Order extrapolation - Bacillariales tolerant

Species	Genus	Order	SPEARgenus	Explanation
Tryblionella hungarica	Tryblionella	Bacillariales	-9999	Order extrapolation - Bacillariales tolerant

Appendix 5. List of chemicals quantified in DSITIA chemical monitoring program by GC-MS.

OC=Organochlorine Pesticides, OP=Organophosphorous Pesticides, HGCM=Herbicides by GCMS, OTHER= Other Pesticides and SP=Synthetic Pyrethroids. Note the listing of chemicals does not imply that a chemical was detected in the samples analysed.

Chemical name (chemical type)	Units	Limit of Reporting
Aldrin (OC)	ug/L	0.1
Chlordane cis (OC)	ug/L	0.1
Chlordane trans (OC)	ug/L	0.1
Chlordene (OC)	ug/L	0.1
Chlordene epoxide (OC)	ug/L	0.1
op-DDD (OC)	ug/L	0.1
pp-DDD (OC)	ug/L	0.1
op-DDE (OC)	ug/L	0.1
pp-DDE (OC)	ug/L	0.1
op-DDT (OC)	ug/L	0.1
pp-DDT (OC)	ug/L	0.1
Total DDT (OC)	ug/L	0.3
Dicofol (OC)	ug/L	0.1
Dieldrin (OC)	ug/L	0.05
Endosulfan alpha (OC)	ug/L	0.05
Endosulfan beta (OC)	ug/L	0.05
Endosulfan Sulphate (OC)	ug/L	0.05
Endosulfan Ether (OC)	ug/L	0.1
Endosulfan Lactone (OC)	ug/L	0.1
Total Endosulfan (OC)	ug/L	0.15
Endrin (OC)	ug/L	0.1
Endrin Aldehyde (OC)	ug/L	0.1
HCB (OC)	ug/L	0.1
HCH alpha (OC)	ug/L	0.1
HCH beta (OC)	ug/L	0.1
HCH delta (OC)	ug/L	0.1

Chemical name (chemical type)	Units	Limit of Reporting
Heptachlor (OC)	ug/L	0.1
Heptachlor Epoxide (OC)	ug/L	0.1
Lindane (OC)	ug/L	0.1
Methoxychlor (OC)	ug/L	0.1
Nonachlor trans (OC)	ug/L	0.1
Oxychlordan (OC)	ug/L	0.1
Azinphos ethyl (OP)	ug/L	0.1
Azinphos methyl (OP)	ug/L	0.1
Bromophos ethyl (OP)	ug/L	0.1
Cadusafos (OP)	ug/L	0.1
Carbophenothion (OP)	ug/L	0.1
Chlorfenvinphos (OP)	ug/L	0.1
Chlorpyrifos (OP)	ug/L	0.1
Chlorpyrifos methyl (OP)	ug/L	0.1
Chlorpyrifos oxon (OP)	ug/L	0.1
Coumaphos (OP)	ug/L	0.1
Diazinon (OP)	ug/L	0.1
Dichlorvos (OP)	ug/L	0.1
Dimethoate (OP)	ug/L	0.1
Ethion (OP)	ug/L	0.1
Ethoprophos (OP)	ug/L	0.1
Etrimphos (OP)	ug/L	0.1
Famphur (OP)	ug/L	0.1
Fenamiphos (OP)	ug/L	0.1
Fenchlorphos (OP)	ug/L	0.1
Fenitrothion (OP)	ug/L	0.1
Fenthion ethyl (OP)	ug/L	0.1
Fenthion methyl (OP)	ug/L	0.1
Isophenphos (OP)	ug/L	0.1
Malathion (OP)	ug/L	0.1
Methidathion (OP)	ug/L	0.1

Chemical name (chemical type)	Units	Limit of Reporting
Mevinphos (OP)	ug/L	0.1
Parathion ethyl (OP)	ug/L	0.1
Parathion methyl (OP)	ug/L	0.1
Phorate (OP)	ug/L	0.1
Phosmet (OP)	ug/L	0.1
Pirimiphos methyl (OP)	ug/L	0.1
Profenofos (OP)	ug/L	0.1
Prothiofos (OP)	ug/L	0.1
Pyrazophos (OP)	ug/L	0.1
Tetrachlorvinphos (OP)	ug/L	0.1
Terbufos (OP)	ug/L	0.1
Diclofop methyl (HGCMS)	ug/L	0.1
Haloxypop-2-etotyl (HGCMS)	ug/L	0.1
Haloxypop methyl (HGCMS)	ug/L	0.1
Metribuzin (HGCMS)	ug/L	0.1
Oxyfluorfen (HGCMS)	ug/L	0.1
Pendimethalin (HGCMS)	ug/L	0.1
Propanil (HGCMS)	ug/L	0.1
Propazine (HGCMS)	ug/L	0.1
Terbutylazine (HGCMS)	ug/L	0.1
Tri-allate (HGCMS)	ug/L	0.1
Trifluralin (HGCMS)	ug/L	0.1
Benalaxyl (OTHER)	ug/L	0.1
Bitertinol (OTHER)	ug/L	0.1
Carbaryl (OTHER)	ug/L	0.1
Dichlorfluanid (OTHER)	ug/L	0.1
Dichloran (OTHER)	ug/L	0.1
Fipronil (OTHER)	ug/L	0.1
Furalaxyl (OTHER)	ug/L	0.1
Metalaxyl (OTHER)	ug/L	0.1
Oxadiazon (OTHER)	ug/L	0.1

Chemical name (chemical type)	Units	Limit of Reporting
Piperonyl Butoxide (OTHER)	ug/L	0.1
Pirimicarb (OTHER)	ug/L	0.1
Procymidone (OTHER)	ug/L	0.1
Propiconazole (OTHER)	ug/L	0.1
Propoxur (OTHER)	ug/L	1
Tebuconazole (OTHER)	ug/L	0.1
Tetradifon (OTHER)	ug/L	0.1
Vinclozolin (OTHER)	ug/L	0.1
Bifenthrin (SP)	ug/L	0.1
lambda-Cyhalothrin (SP)	ug/L	0.1
Cyfluthrin (SP)	ug/L	0.1
Cypermethrin (SP)	ug/L	0.1
Deltamethrin (SP)	ug/L	0.1
Fluvalinate (SP)	ug/L	0.1
Phenothrin (SP)	ug/L	0.1
Tetramethrin (SP)	ug/L	0.1
Transfluthrin (SP)	ug/L	0.1
Permethrin (SP)	ug/L	0.1
Fenvalerate (SP)	ug/L	0.1
Allethrin (SP)	ug/L	0.1
Bioresmethrin (SP)	ug/L	0.1