

# Reef Rescue Research & Development

## Technical Report

Pesticide dynamics in the Great Barrier Reef  
catchment and lagoon: grazing, bananas and grain:  
Herbicide persistence in the marine environment  
**RRRD038**



**Andrew P Negri, Phil Mercurio, Florita Flores,  
Geoff Eaglesham and Jochen F Mueller**

entox

national research centre for  
environmental toxicology



Australian Government  
Department of the Environment

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OF MARINE SCIENCE

**Pesticide dynamics in the Great Barrier Reef  
catchment and lagoon: grazing, bananas and  
grain: *Herbicide persistence in the marine  
environment***  
**(Project RRRD038)**

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The Reef Rescue Research and Development Program (Reef Rescue R&D) was funded under the Australian Government's Caring for our Country program, and consisted of 18 projects. The Reef Rescue R&D is designed to improve our understanding of the link between land management practices and environmental impacts, and improve water quality across the Great Barrier Reef by informing on-ground projects to reduce the amounts of nutrients, pesticides and sediments reaching the Reef from agricultural lands. The project was extended for one year in 2013-14 under the Australian Government Reef Programme.

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Cover photographs: Top Left – Loading herbicides into flask for persistence experiments; Top Right - Flasks used for herbicide degradation experiments in standard incubator tests; Bottom Left - Outdoor pond tanks; Bottom Right – Initiation of indoor experiments. Images supplied by Phil Mercurio and Andrew Negri, Australian Institute of Marine Science.

This report is available from the Reef Rescue R&D website: <http://www.reefrescueresearch.com.au>

## Executive Summary

Runoff containing pesticides from agricultural land is recognised as a potential threat to inshore habitats of the Great Barrier Reef (GBR), with the photosystem II (PS) herbicides, including Diuron and Atrazine being the most frequently detected pesticides in marine waters of the GBR. These herbicides act by inhibiting photosynthesis in terrestrial weeds and can impact non-target marine species including corals and seagrass in the same way. The potential for pesticides to move from paddock to the reef depends on their individual properties, including their persistence and solubility. Understanding the persistence of herbicides is critical to better parameterize and validate predictive risk models and to better inform the management of application methods and rates of pesticides in the GBR catchments to reduce runoff.

This was the first study to quantify the seawater persistence of priority herbicides detected in the GBR lagoon. Standard Flask *Experiment 1A* revealed extremely slow degradation of Diuron, Atrazine, Hexazinone, Tebuthiuron, Simazine, and Ametryn in the absence of light and sediments, with all herbicides exhibiting half-lives of between 657 and 1016 days. *Experiment 2* was conducted in outdoor ponds with unfiltered coastal water and treatments included the presence and absence of light and sediments. These conditions more closely mimic coastal habitats in the GBR lagoon than flask experiments in incubators. Under the most environmentally relevant conditions (light + sediments) the order of persistence was Metolachlor (half-life,  $t_{1/2}$  = 32 d) < Atrazine (107 d) < Diuron (139 d) < Hexazinone (201 d) < 2,4-D (288 d) < Tebuthiuron (944 d). The presence of coastal sediments consistently reduced the persistence (1.1 – 6.3-fold) of herbicides, especially in moderate sunlight. Moderate sunlight also reduced the persistence of Diuron, Atrazine, Hexazinone, Tebuthiuron and Metolachlor in the presence of sediments. 2,4-D was a notable exception and was far more persistent in the light. Changes in persistence were most likely due to the influence of light and sediments on microbial populations.

The long persistence of herbicides in seawater in the pond *Experiment 2*, demonstrates that negligible herbicide degradation is likely in flood plumes lasting for weeks and that herbicides reaching the GBR lagoon may persist there for many months to years. These results help explain the year-round detection of herbicides in the GBR lagoon. This reliable persistence data for priority herbicides detected in the GBR lagoon is now available to managers, regulators and industry to enable (in combination with exposure and toxicity data) more effective and targeted management of these agricultural contaminants and develop more accurate spatial risk assessment models for coral reefs and seagrass beds of the GBR. Further work is needed to quantify the fate of dozens of other herbicides, fungicides and insecticides, as well urban and industrial contaminants detected in the GBR lagoon.

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## Acronyms

|                |                                                                            |
|----------------|----------------------------------------------------------------------------|
| <b>AIMS</b>    | Australian Institute of Marine Science                                     |
| <b>DSEWPac</b> | Department of Sustainability, Environment, Water, Population and Community |
| <b>ENTOX</b>   | National Research Centre for Environmental Toxicology                      |
| <b>GBR</b>     | Great Barrier Reef                                                         |
| <b>GBRMPA</b>  | Great Barrier Reef Marine Park Authority                                   |
| <b>JCU</b>     | James Cook University                                                      |
| <b>NRM</b>     | Natural Resource Management                                                |
| <b>NERP</b>    | National Environmental Research Program                                    |
| <b>OECD</b>    | Organisation for Economic Cooperation and Development                      |
| <b>QDAFF</b>   | Queensland Department of Agriculture, Fisheries and Forestry               |
| <b>RRRC</b>    | Reef and Rainforest Research Centre Limited                                |
| <b>RRRD</b>    | Reef Rescue Research & Development                                         |
| <b>UQ</b>      | University of Queensland                                                   |

## Abbreviations

|                             |                  |
|-----------------------------|------------------|
| <b><math>T_{1/2}</math></b> | Half Life (days) |
|-----------------------------|------------------|

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# Introduction

## ***Background***

Runoff containing pesticides from agricultural land is recognised as a potential threat to nearshore habitats of the Great Barrier Reef (GBR) (Lewis et al., 2009; Brodie et al., 2013). The photosystem II (PS) herbicides, including Diuron and Atrazine, are the most frequently detected pesticides in rivers, marine waters, sediments and biota of the GBR (Haynes et al., 2000; Lewis et al., 2009; Davis et al., 2012). The total load of PSII herbicides entering the GBR lagoon is estimated to be at least 30,000 kg per year (Kroon et al., 2012). These herbicides act by inhibiting photosynthesis in terrestrial weeds and can impact non-target marine species including corals and seagrass in the same way (Jones and Kerswell, 2003; Flores et al., 2013). The Reef Water Quality Protection Plan (Reef Plan, 2013) outlines water quality targets to reduce the runoff of pesticides with an ultimate goal of improving ecosystem resilience in the GBR. The concentrations and loads of pesticides in runoff are monitored and reported annually as part of this program.

The potential for pesticides to move from paddock to the reef depends on their individual properties, including their persistence and solubility. A quantitative mass balance approach of individual pesticides is essential to better parameterise/validate predictive models such as Source Catchments to improve the accuracy of load calculations. This information is critical in the management of application methods and rates of pesticides in the GBR catchments to reduce runoff and to inform/validate the modelling process within the Reef Rescue monitoring and evaluation program (Reef Plan, 2009).

This sub-project contributes to the collaboration of research scientists, growers and industry stakeholders in *RRRD038* to address the major unknowns of pesticide dynamics in the GBR catchment area. The persistence (half-lives) of PSII herbicides in the GBR catchment area and lagoon is a substantial research gap to evaluate the risk of pesticide residues in the GBR lagoon. Here we undertake seawater flask and pond trials to examine the persistence of key dissolved and particle-associated pesticides of greatest concern in the GBR lagoon. These results will provide a critical measure of pesticide persistence in the GBR catchment area and lagoon, allowing for much improved risk assessments to be made on pesticide exposure in the GBR.

## ***Project Objective***

The key objective of this study is to quantify the persistence in seawater of key pesticides detected in the Great Barrier Reef lagoon.

## Methods

### *Herbicide Persistence (Marine Component)*

A series of two long-term laboratory flask experiments and an outdoor pond experiment were conducted to quantify the persistence of common herbicides in tropical seawater. The Caring for our Country experiments (1A and 2) are integrated with a related NERP Tropical Ecosystems-funded Project 4.2 “The chronic effects of pesticides and their persistence in tropical waters” (experiment 1B) to provide a more comprehensive dataset for herbicide persistence under a range of environmental conditions than was originally planned. Table 1 provides an outline of the herbicides and the experimental conditions in each experiment.

**Table 1: Details of three degradation experiments investigating herbicide degradation.**

| Experiment            | 1A. Flask: filtered seawater<br>Caring for our Country                                                                                                                                               | 1B. Flask: unfiltered<br>seawater<br>NERP                                                                                                                                                                    | 2. Outdoor pond<br>Caring for our Country                                                                                                                                   |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Experimental units    | 3 replicate flasks                                                                                                                                                                                   | 4 replicate flasks                                                                                                                                                                                           | 3 replicate ponds                                                                                                                                                           |
| Herbicides            | <ul style="list-style-type: none"> <li>• Control</li> <li>• PSII mix (Ametryn, Atrazine, Diuron, Hexazinone, Simazine, Tebuthiuron)</li> <li>• PSII mix (as above) with mercuric chloride</li> </ul> | <ul style="list-style-type: none"> <li>• Control</li> <li>• 2,4-D</li> <li>• Atrazine</li> <li>• Diuron</li> <li>• Glyphosate</li> <li>• Hexazinone</li> <li>• Metolachlor</li> <li>• Tebuthiuron</li> </ul> | <ul style="list-style-type: none"> <li>• Control</li> <li>• PSII mix (Diuron, Atrazine, Tebuthiuron, Hexazinone)</li> <li>• Herbicide Mix 2 (2,4-D, Metolachlor)</li> </ul> |
| Seawater              | 0.45 µm filtered                                                                                                                                                                                     | 20 µm filtered                                                                                                                                                                                               | Unfiltered                                                                                                                                                                  |
| Light conditions      | Dark only                                                                                                                                                                                            | Dark and low light (40 µE)                                                                                                                                                                                   | Dark and Light (ambient shaded maximum 600-700 µE)                                                                                                                          |
| Temperature           | 25°C                                                                                                                                                                                                 | 25°C and 31°C                                                                                                                                                                                                | Mean 28 ± 3 (SD)<br>Range 21- 36°C                                                                                                                                          |
| Additional treatments |                                                                                                                                                                                                      |                                                                                                                                                                                                              | Sediments present in half of the tanks                                                                                                                                      |
| Expected duration     | 300 days                                                                                                                                                                                             | 12 months                                                                                                                                                                                                    | 12 months                                                                                                                                                                   |
| Completion date       | 19/12/2012                                                                                                                                                                                           | 15/5/2013                                                                                                                                                                                                    | 22/8/2013                                                                                                                                                                   |

#### *Experiment 1: Standard flask with filtered seawater (Caring for our Country)*

The first flask experiment was designed to quantify the persistence of 6 PSII herbicides in seawater in the absence of sediments and suspended particulates (Table 1). The experimental procedure was adapted from OECD 306 guideline for testing of chemicals: biodegradability in seawater (OECD, 1992). Near shore coastal seawater was collected at the Australian Institute of Marine Science (AIMS), Cape Cleveland, QLD and filtered to 0.45 µm. The filtered seawater was added to 500 mL Erlenmeyer flasks to a final volume of 300 mL and sample treatments were spiked with herbicides to a final concentration of 10 µg/L each (Table 1). Each flask was stoppered with autoclaved cotton bungs to allow for aerobic conditions. The pilot degradation experiment was initially designed to run for a duration of 6

months but this was extended to 300 days to allow for additional degradation of the herbicides under the experimental conditions.

Triplicate flasks were housed in an Innova 44, incubator shaker series at 25°C and 100 rpm in the dark. Treatments were as follows: control, herbicide mixture (ametryn, atrazine, diuron, hexazinone, simazine, tebuthiuron), and herbicide mixture with mercuric chloride (non-biological degradation treatment). Sample treatment flasks were weighed before sampling to monitor evaporation losses for concentration adjustments. An aliquot of 2 mL sample was pipetted into labeled 4 mL amber glass vials and spiked with 10  $\mu$ L of a surrogate standard, d5-atrazine. The final concentration of the surrogate standard was 5  $\mu$ g/L. The samples were stored at -20°C and filtered prior to analysis.

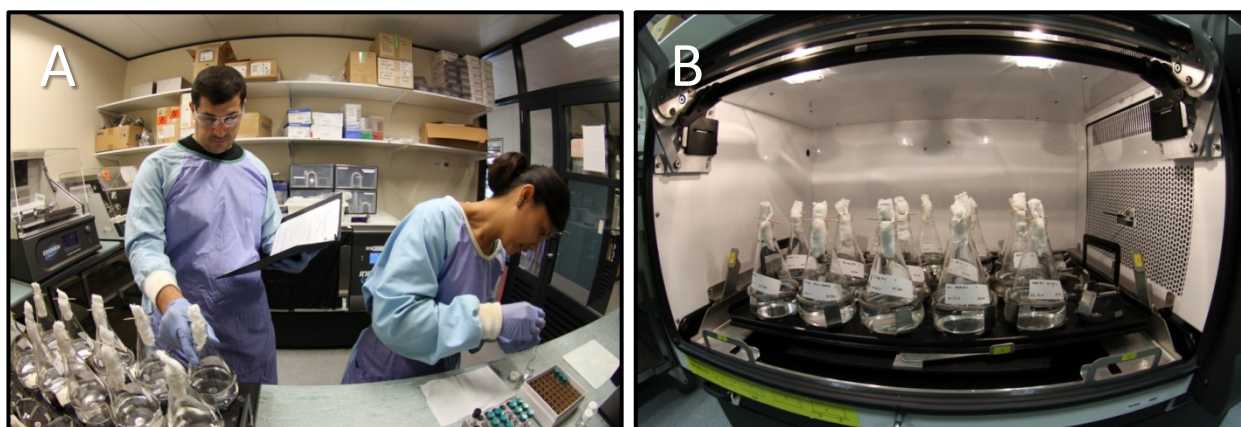


Figure 1: Flask experiment 1: (A) preparation of flasks and (B) incubator shaker used to test the persistence of herbicides under standard conditions. Images P. Mercurio.

#### ***Experiment 1B: Standard flask with unfiltered coastal seawater (NERP Project 4.2)***

The second flask experiment was designed to quantify the persistence of herbicides in coastal seawater at two temperatures and in the presence and absence of low light expected during flood plumes (Fig. 2). This experiment is primarily NERP-funded (Project 4.2) but is included in the Methods section for integration purposes (see Table 1).



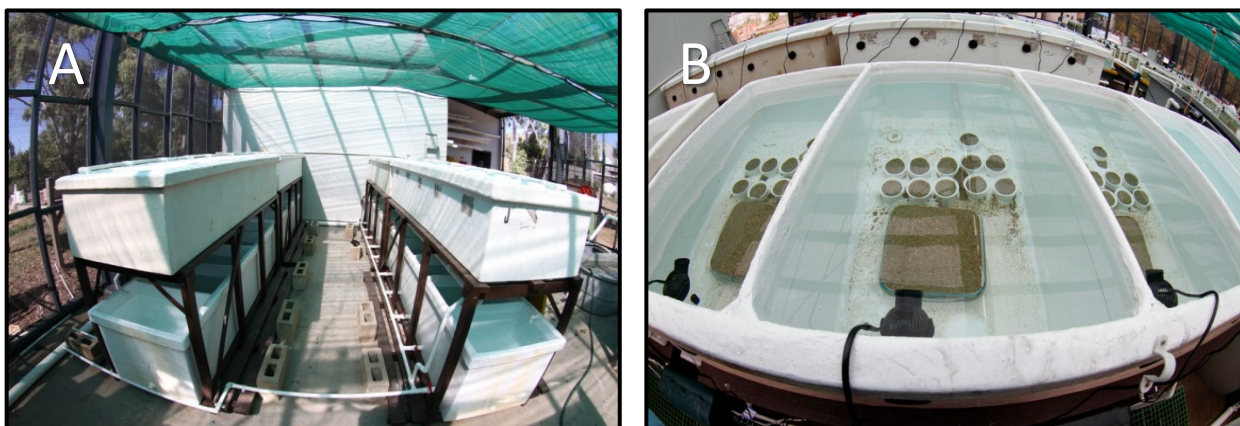
Figure 2: Flask experiment in incubator shakers to test the persistence of herbicides under standard conditions. Image P. Mercurio.

***Experiment 2: Outdoor pond experiments (Caring for our Country)***

The pond experiment was designed to measure the degradation of the herbicides under more natural temperature, light/dark conditions, and with the inclusion of coastal sediments in half the ponds (Table 1). Experiment fibreglass troughs were designed to incorporate 40 individual ponds (which hold 120 L each) (Fig. 3A). Troughs were exposed to several conditioning periods with freshwater and seawater prior to the final additions of herbicide solutions. Herbicide treatment/replicate positions were randomized and the dark treatments were positioned directly below the light treatment of the same herbicide condition replicate. The troughs were housed in an outdoor experimental facility with a shade cloth enclosure. All individual ponds had a cover to reduce evaporation losses, clear acrylic (6 mm thickness) for the top light treatments and dark foam rubber (6 mm thickness) for the bottom dark treatments. The light intensity was recorded for the experiment duration through a series of Hobo data (light/temperature) loggers. At mid-day with 70% shade cloth, the light intensity readings were up to 600-700  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Pond water was circulated using Turbelle Nanostream pumps (Tunze GmbH) in each individual pond.

Sediments for the pond degradation experiment were collected during low tide from Cockle Bay, Magnetic Island, Queensland. The sediments were prepared one week prior to use by sieving (> 2 mm removed), thorough mixing and conditioning in shallow trays placed in two 1000 L tanks with 20  $\mu\text{m}$  filtered and aerated seawater. Half of the sediment was held in a blackened/covered trough and the other half was held in shaded sunlight. This conditioning process allowed for a transition in the dark trays from autotrophic to heterotrophic only communities prior to the start of the experiment. Sediments were distributed into two types of containers: glass dishes and small round baking dishes (Fig. 3B). The glass dishes (1.8 L, 25 cm x 22 cm x 5 cm) held approximately 3 kg of sediment each. The small baking dishes (ramekins; 6.5 cm diameter) held approximately 80 g sediment and were used for reproducible time point sampling. Each pond sediment treatment held a single glass dish and 11 round dishes, allowing for 11 sampling sediment sampling time points. A total of 6 herbicides were included in this experiment, each with an initial concentration of 10  $\mu\text{g/L}$  (Table 1).

Water samples were treated as per *Experiment 1A*. The sediment dishes were removed from the ponds using treatment-dedicated long aluminum tongs. The sediment samples were mixed/homogenised and then transferred to 50 mL falcon tubes and weighed to approximately 55 grams. Both wet and dry weights were taken for each sediment sample before commencing extraction. The samples were centrifuged for 10 min at 3000 rpm, excess water removed and stored in the freezer for later analyses (OECD, 2000).



**Figure 3: Outdoor ponds: (A) a series of 40 x 120 L fiberglass troughs in an outdoor shadehouse with approximately two-thirds shading from full sunlight; (B) view inside ponds with discrete sediment containers for periodic sampling. Images P. Mercurio.**

### ***Herbicide analyses***

The herbicide and degradation product concentrations were determined by HPLC-MS/MS using an AB/Sciex API5500Q mass spectrometer (AB/Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface and coupled to a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan). Column conditions were as follows: Phenomenex Synergi Fusion RP column (Phenomenex, Torrance, CA) 4  $\mu\text{m}$  50 x 2.0 mm, 45°C, with a flow rate of 0.4 mL/min. The column was conditioned prior to use and for analyte separation required a linear gradient starting at 8% B for 0.5 minutes, ramped to 100% B in 8 minutes then held at 100% for 2.0 minutes followed by equilibration at 8% B for 2.5 minutes. (A = 1% methanol in HPLC grade water, B = 95% methanol in HPLC grade water, both containing 0.1% acetic acid). The mass spectrometer was operated in the positive ion, multiple reaction-monitoring mode (MRM) using nitrogen as the collision gas.

Positive samples were confirmed by retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Samples were reported as positive if the two transitions were present, retention time was within 0.15 minutes of the standard and the relative intensity of the confirmation transition was within 20% of the expected value. The value reported was that for the quantitation transition. The limit of detection for this method was typically less than 0.1  $\mu\text{g/L}$ , with a reporting limit of 0.5  $\mu\text{g/L}$  in the sample. Response was linear to at least 20  $\mu\text{g/L}$  which is within the range of the samples. Sample sequences were run with a standard calibration at beginning and end of sequence with additional mid-range standards run every 10 samples.

## Results

### *Herbicide Persistence (Marine Component)*

#### *Experiment 1A: Standard flask with filtered seawater*

All six PSII herbicides exhibited very slow degradation in the dark in 0.45  $\mu\text{m}$ -filtered seawater, with none of the herbicide concentrations dropping by more than 20% of their original concentrations by the end of the experiment at 300 days (Fig. 4, Table 2). Half-lives were estimated from the first order kinetic equations (see Fig. 5). Simazine degraded most rapidly, with an estimated half-life ( $T_{1/2}$ ) of  $657 \pm 129$  days, while Hexazinone was the most persistent ( $T_{1/2} = 922 \pm 264$  days) (Table 2).

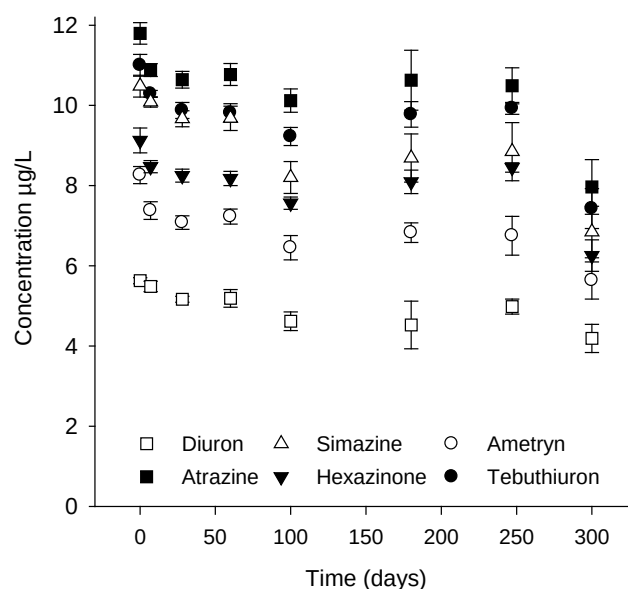
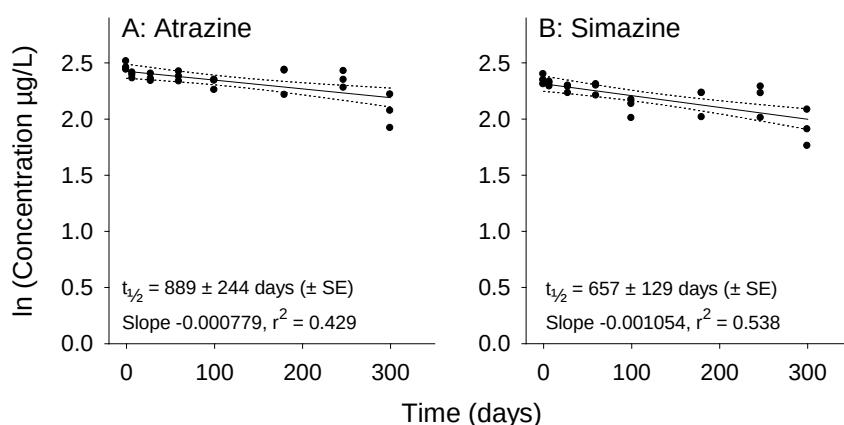


Figure 4: Herbicide concentrations ( $\mu\text{g/L}$ ) in Flask *Experiment 1*.

The persistence of herbicides was longer in the sterile conditions of the mercuric chloride treatment (16% longer for Diuron to 101% longer for Simazine) (Table 2). Only the persistence of Hexazinone was unaffected by the removal of all microbial activity.

Table 2: *Experiment 1A*: Estimated half-lives ( $T_{1/2}$ ) of six photosystem II (PSII) herbicides (days) in 0.45  $\mu\text{m}$  filtered seawater in the dark.

| Treatment                          | Herbicide half-life ( $T_{1/2}$ , days) |                |                |                |                |                |
|------------------------------------|-----------------------------------------|----------------|----------------|----------------|----------------|----------------|
|                                    | Diuron                                  | Atrazine       | Hexazinone     | Tebuthiuron    | Simazine       | Ametryn        |
| Filtered water                     | $870 \pm 233$                           | $889 \pm 244$  | $1016 \pm 326$ | $922 \pm 264$  | $657 \pm 129$  | $853 \pm 223$  |
| Filtered water + mercuric chloride | $1009 \pm 150$                          | $1260 \pm 237$ | $1039 \pm 160$ | $1133 \pm 190$ | $1322 \pm 262$ | $1220 \pm 222$ |



**Figure 5: Natural log of (A) Atrazine and (B) Simazine concentrations ( $\mu\text{g/L}$ ) vs time (days) in *Flask Experiment 1*.  $N = 3$  flasks for each time point, dotted lines represent 95% confidence intervals for each slope.  $\ln(C) = a + k_1t$ , where  $C$  is the concentration,  $a$  is the intercept,  $t$  is time and  $k_1$  the pseudo first order rate constant. The half-life can then be calculated from the equation  $t_{1/2} = 0.693/k_1$ .**

### **Experiment 2: Outdoor ponds**

The pond experiment ran successfully for 12 months with most of the herbicides exhibiting long persistence under both light and dark conditions. Examples of the absolute herbicides concentrations can be found in Fig. 6. The degradation generally followed pseudo first order kinetics with regression of the  $\ln(\text{concentration})$  vs time exhibiting strong correlations (only 3 of the 24 treatments exhibited  $r^2$  values less than 0.7, Table A4). The half-lives were calculated from the slope of the natural logs of these concentrations (See examples Fig. 7-9).

**Table 3: Estimated half-lives of common herbicides (days) in outdoor pond experiments. Herbicides were exposed to four combinations of environmental conditions comprising of the presence and absence of light and sediments.**

| Light | Sediment | Herbicide half-life ( $T_{1/2}$ , days) |              |                |                |                |              |
|-------|----------|-----------------------------------------|--------------|----------------|----------------|----------------|--------------|
|       |          | Diuron                                  | Atrazine     | Hexazinone     | Tebuthiuron    | 2,4-D          | Metolachlor  |
| +     | -        | $404 \pm 16$                            | $330 \pm 32$ | $1186 \pm 73$  | $3330 \pm 419$ | $1826 \pm 383$ | $94 \pm 9$   |
| +     | +        | $139 \pm 6$                             | $107 \pm 7$  | $201 \pm 18$   | $944 \pm 42$   | $288 \pm 12$   | $32 \pm 3$   |
| -     | -        | $499 \pm 31$                            | $300 \pm 13$ | $1994 \pm 207$ | $1766 \pm 188$ | $59 \pm 15$    | $147 \pm 13$ |
| -     | +        | $279 \pm 7$                             | $201 \pm 6$  | $1025 \pm 51$  | $1474 \pm 106$ | $56 \pm 6$     | $103 \pm 5$  |

In unfiltered coastal water and in the absence of light and sediments the PSII herbicides Diuron, Atrazine, Hexazinone, and Tebuthiuron exhibited half-lives ranging from  $300 \pm 13$  (SE) days (Atrazine) to  $1994 \pm 201$  days (Tebuthiuron) (Table 3). The half-lives of the four PSII herbicides were reduced by between 17% (Tebuthiuron) and 49% (Hexazinone) in the presence of coastal sediments. The presence of light had no little effect on the persistence of Atrazine ( $\sim 10\%$  more persistent) but caused 19% and 41% reductions in the persistence of Diuron and Hexazinone, respectively, and almost doubled the persistence of Tebuthiuron in the absence of sediments. The most rapid degradation was observed in the presence of light and sediments:  $T_{1/2} = 139 \pm 6$  days for Diuron (Fig. 5B),  $107 \pm 7$  days for Atrazine,  $201 \pm 18$  days for Hexazinone, and  $994 \pm 42$  days for Tebuthiuron (Table 3).

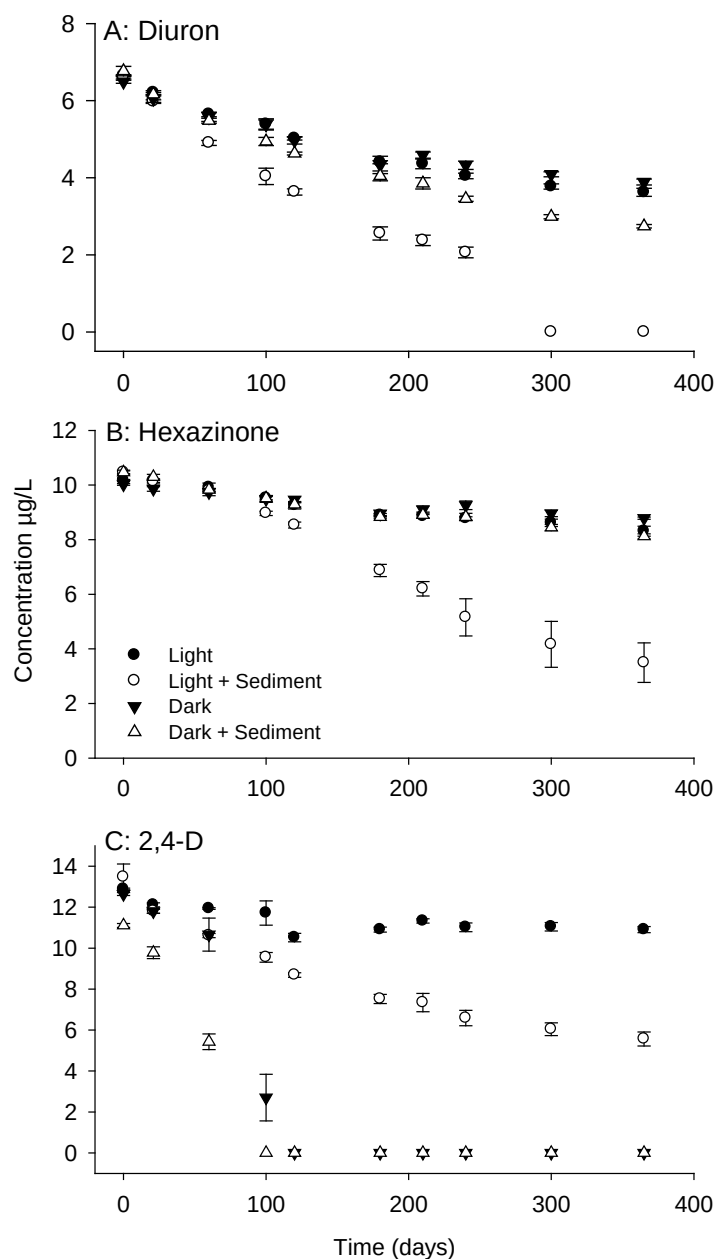
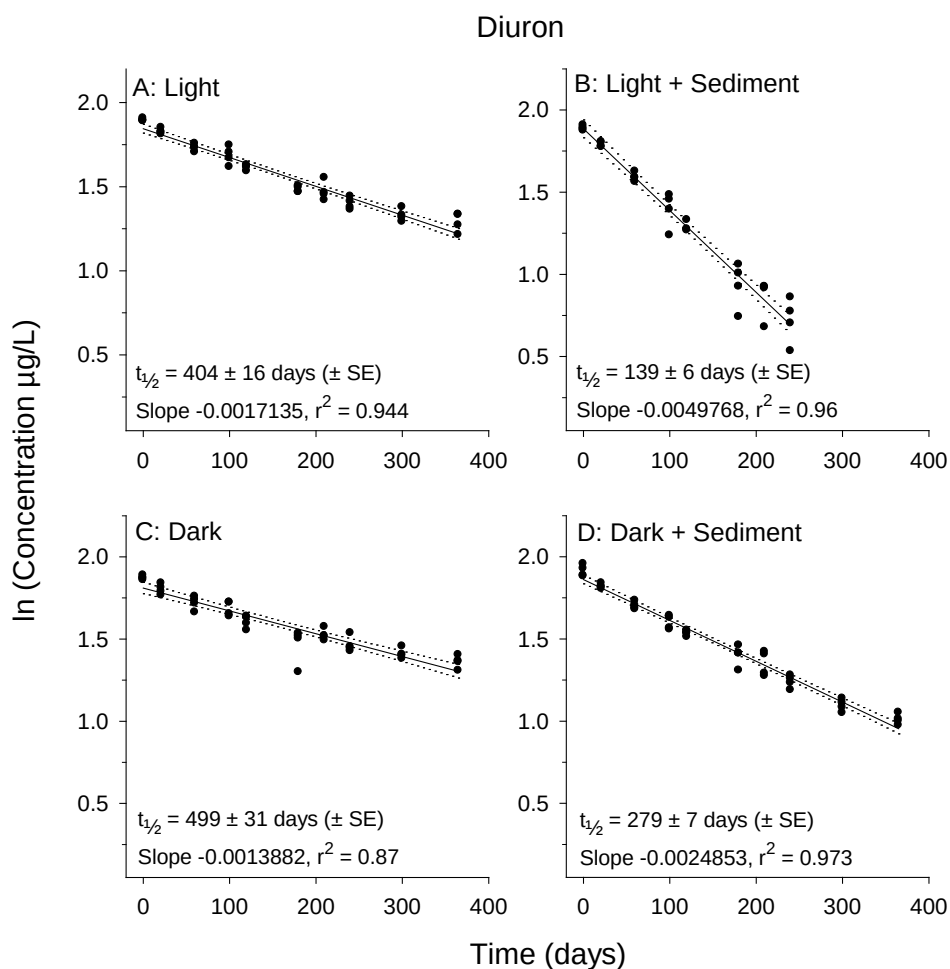


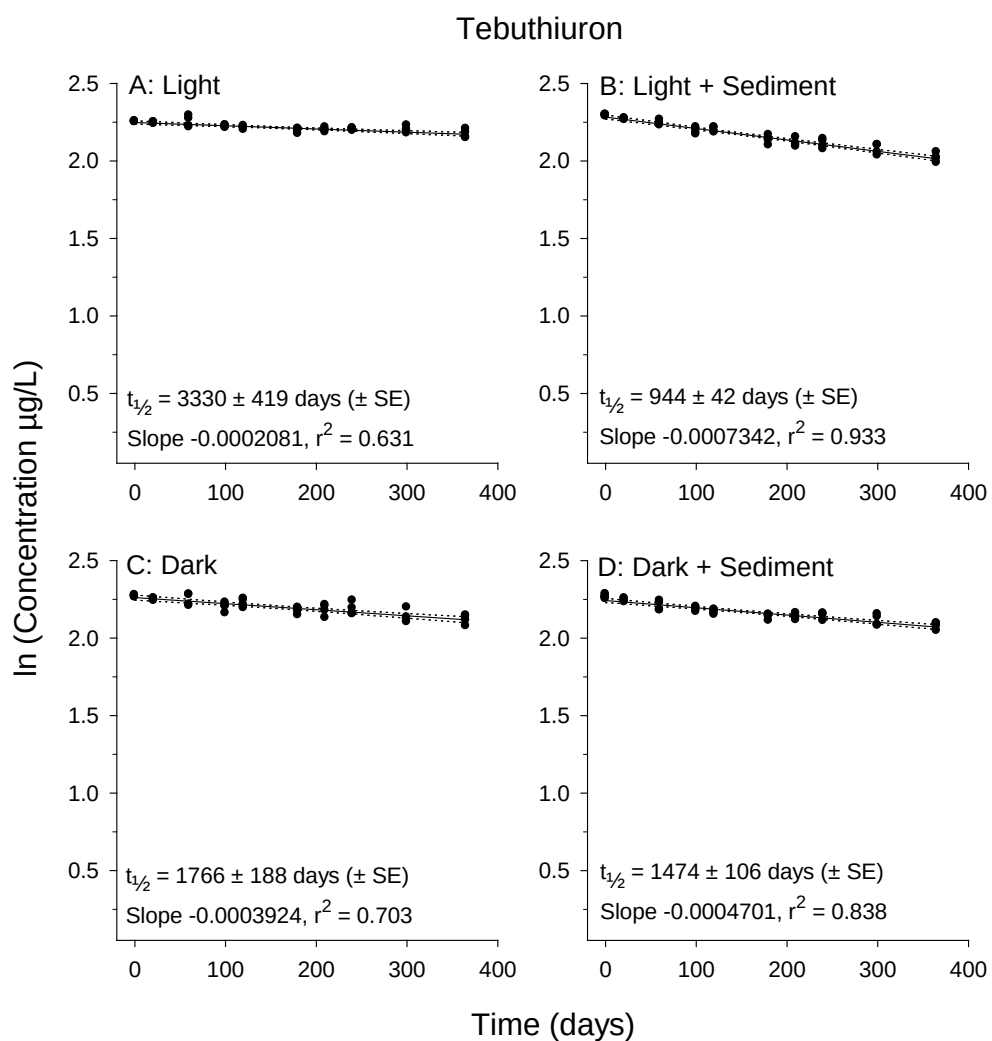
Figure 6: Herbicide concentrations (µg/L) in Pond Experiment 2: (A) Diuron, (B) Hexazinone and (C) 2,4-D.

A similar pattern of degradation was observed for the long-chain fatty acid inhibitor Metolachlor which degraded most rapidly in the light and the presence of sediment ( $T_{1/2} = 32 \pm 3$  days) and most slowly in the absence of light and sediment  $T_{1/2} = 147 \pm 13$  days (Table 3).

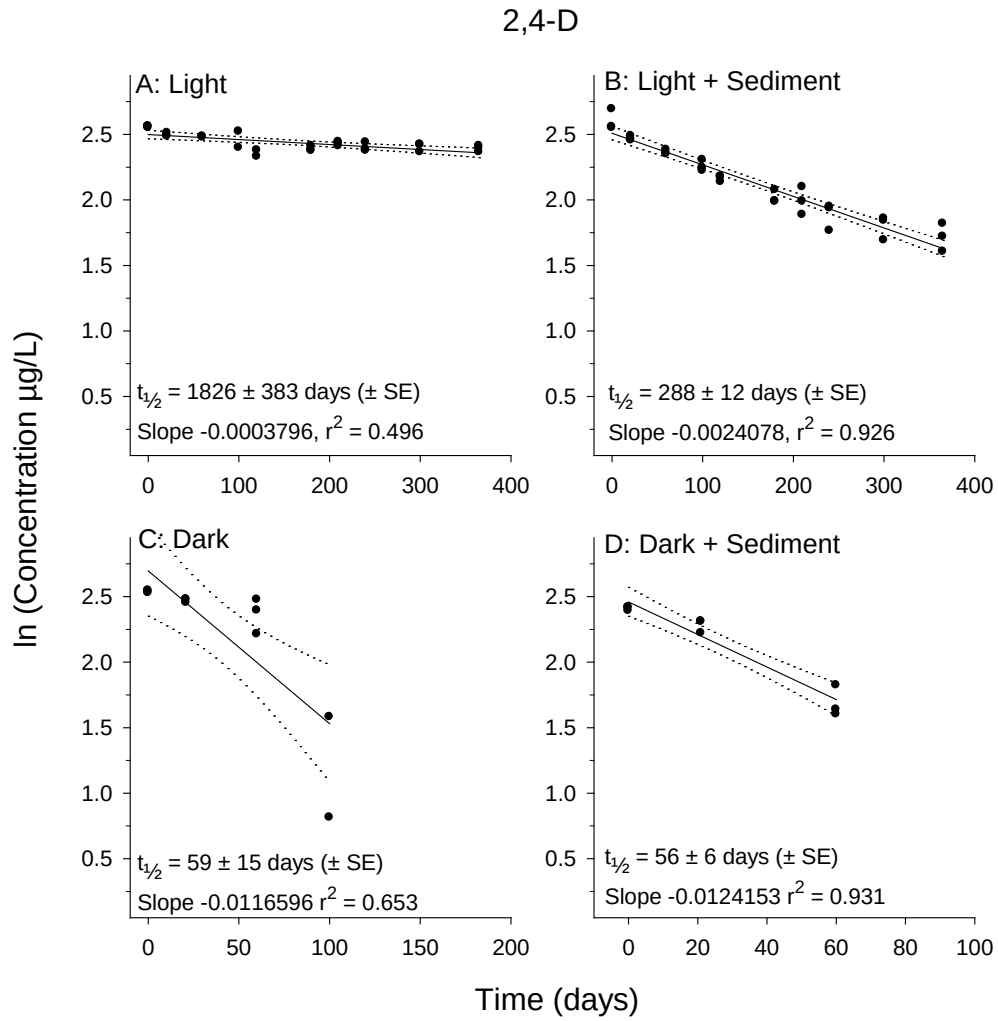
Conversely, the growth regulator 2,4-D degraded most rapidly in the dark with little impact of sediment under these conditions:  $T_{1/2} = 59 \pm 15$  (no sediment) to  $56 \pm 6$  days (sediment) (Fig. 7, Table 3). This herbicide was more persistent in the presence of light with a much longer half-life of  $1826 \pm 383$  days projected in the absence of sediment and  $288 \pm 12$  days in the presence of sediment (Table 3).



**Figure 7: Natural log of Diuron concentrations ( $\mu\text{g/L}$ ) and half-life ( $t_{1/2}$ ) for each treatment in Pond *Experiment 2*: (A) Light, (B) light with sediment, (C) dark and (D) dark with sediment.  $N = 4$  tanks for each time point, dotted lines represent 95% confidence intervals for each slope.  $\ln(C) = a + k_1t$ , where  $C$  is the concentration,  $a$  is the intercept,  $t$  is time and  $k_1$  the pseudo first order rate constant. The half-life can then be calculated from the equation  $t_{1/2} = 0.693/k_1$ .**



**Figure 8: Natural log of Tebuthiuron concentrations ( $\mu\text{g/L}$ ) and half-life ( $t_{1/2}$ ) for each treatment in Pond *Experiment 2*: (A) Light, (B) light with sediment, (C) dark and (D) dark with sediment.  $N = 4$  tanks for each time point, dotted lines represent 95% confidence intervals for each slope.**



**Figure 9:** Natural log of 2,4-D concentrations ( $\mu\text{g/L}$ ) and half-life ( $t_{1/2}$ ) for each treatment in Pond *Experiment 2*: (A) Light, (B) light with sediment, (C) dark and (D) dark with sediment.  $N = 3$  tanks for each time point, dotted lines represent 95% confidence intervals for each slope.

## Discussion

### ***Herbicide Persistence (Marine Component)***

#### ***Experimental conditions and the degradation of herbicides***

The Flask *Experiment 1A* was designed as a preliminary range finder to help design the main *Experiment 2* to be conducted in outdoor pond conditions. *Experiment 1A* closely followed OECD-style “simulation” protocols (low herbicide concentrations, no light and no sediments or artificial bacterial inoculum) and the results can be more readily compared with other “standard” protocols. The pond experiment, on the other hand, used unfiltered coastal water and half the treatments were exposed to sediments and moderate sunlight to better mimic conditions in coastal waters. Differences in herbicide persistence between these experiments is expected as: (i) light may enhance degradation; (ii) sediments and less filtered water in the pond experiment will support different microbial communities and (iii) conditions in the pond experiment are likely to include a greater abundance and diversity of carbon sources which may reduce microbial consumption of the herbicides.

#### ***Influence of sediments on the persistence of herbicides (Experiment 2)***

There was a dramatic increase in degradation of herbicides in the presence of coastal sediments. In the dark the ratio of  $t_{1/2} (- \text{sediments}) / t_{1/2} (+ \text{sediments})$  was 1.1 for 2,4-D (indicating 10% more rapid degradation in the presence of sediments and this ratio was highest (1.9) for Hexazinone which demonstrates this herbicide degrades almost twice as rapidly in the presence of sediments (Table 4). The impact of sediments was even more pronounced in the presence of light where all herbicides degraded at least 2.9-fold more rapidly in the presence of sediments (Table 4).

**Table 4: Experimental. Estimated ratio of half-lives of herbicides in the absence/presence of sediments in pond experiments.**

|       | Diuron | Atrazine | Hexazinone | Tebuthiuron | 2,4-D | Metolachlor |
|-------|--------|----------|------------|-------------|-------|-------------|
| Light | 2.9    | 3.1      | 5.9        | 3.5         | 6.3   | 2.9         |
| Dark  | 1.8    | 1.5      | 1.9        | 1.2         | 1.1   | 1.4         |

The presence of sediments is likely to increase the diversity and possibly the abundance of bacteria capable of utilising the herbicides as a source of carbon. It is possible that a small proportion of the herbicides may have become bound to the sediments (Davis et al., 2012) and not sampled (from the water column) during the experiment, but this is not likely to have contributed greatly to the rate of degradation as there was no initial drop in herbicide concentrations (indicating binding) in the first days of the experiment.

#### ***Influence of light on the persistence of herbicides (Experiment 2)***

The presence of moderate levels of natural light on a daily basis generally increased the rates of degradation (Table 5), ratios of  $t_{1/2} (\text{dark}) / t_{1/2} (\text{light}) > 1$ . This was particularly true for

Hexazinone which degraded 5-fold more rapidly in the presence of light when sediments were also present (Table 5). However in the absence of sediments Atrazine and Tebuthiuron degraded more slowly in the dark. 2,4-D was an even more noteworthy exception, degrading 5-fold more slowly in the light when sediments were present and this effect was further exaggerated in the absence of sediments (Table 5).

**Table 5: Estimated ratio of half-lives of herbicides in the dark/light in pond experiments.**

|              | Diuron | Atrazine | Hexazinone | Tebuthiuron | 2,4-D | Metolachlor |
|--------------|--------|----------|------------|-------------|-------|-------------|
| Sediments    | 2.0    | 1.9      | 5.1        | 1.6         | 0.2   | 3.2         |
| No Sediments | 1.2    | 0.9      | 1.7        | 0.5         | 0.032 | 1.6         |

Light may influence the persistence of herbicides by:-

1. Favoring different (potentially phototrophic) microbial communities that in turn increases or decreases degradation.
2. Changing the abundance of alternative carbon sources which increases or decreases microbial degradation
3. Photodegradation by the moderately high levels of light (up to 700  $\mu\text{E}$ ), although little UV light is expected to penetrate into the tanks.

### ***Comparison of herbicide persistence in seawater with other studies***

There have been few other studies on the persistence of relevant herbicides in seawater. Atrazine has been the focus of 4 previous studies (Table 6). In light and in the absence of sediments we observed a half-life of 330 days (reduced to 107 days by the inclusion of sediment). This persistence is longer than reported at 20 – 40°C in light conditions (Konstantinou et al., 2001; Navarro et al., 2004a; Navarro et al., 2004b) with the fourth study not finding any degradation by 42 d (Meakins et al., 1995). The only similar study on Diuron found no degradation after 42 days which is relatively consistent with the long persistence in our study under natural light (404 d and 139 d in the absence and presence of sediment respectively).

The persistence of 2,4-D in seawater in the light was reported to be as rapid as  $t_{1/2} = 11$  days (Dąbrowska et al., 2006), much shorter than the 1826 d under similar conditions in our experiment. However, in the dark 2,4-D was reported to have a similar persistence of  $t_{1/2} = 31$  days (Dąbrowska et al., 2006) as we found under similar conditions (Table 3). The results of the present study greatly increase the persistence information available to regulators, managers and industry for application in risk assessment models for relevant herbicides in the GBR lagoon.

**Table 6: Half-lives of relevant herbicides (days) in seawater in from other studies.**

| Herbicide | Half-life (days)        | Initial conc. | Dark (D)<br>Light (L)<br>Temperature (T)    | Filtered?             | Comments                                                                                     | Reference                   |
|-----------|-------------------------|---------------|---------------------------------------------|-----------------------|----------------------------------------------------------------------------------------------|-----------------------------|
| 2,4-D     | L = 11 d<br>D = 31 d    | 2 mg/L        | L = natural light<br>D = dark<br>T = 20°C   | No                    |                                                                                              | (Dąbrowska et al., 2006)    |
| Atrazine  | L = 79 d<br>D = 206 d   | 5 mg/L        | L = light (8 h sun)<br>D = dark<br>T = 20°C | No                    | Also looked at river and groundwater degradation and included 2 additional herbicides        | (Navarro et al., 2004a)     |
| Atrazine  | S = 79 d<br>T = 65 d    | 5 mg/L        | S = 20°C, light<br>T = 40°C, light          | No                    | Also looked at river and groundwater degradation and included 2 additional herbicides        | (Navarro et al., 2004b)     |
| Atrazine  | No degradation for 20 d | 20 µg/L       | L = unknown intensity<br>T = 20°C           | 0.45µm and unfiltered | Noted absence of degradation compounds. Included 2 degradation products.                     | (Meakins et al., 1995)      |
| Atrazine  | L = 56 d                | 8-9 mg/L      | L = natural<br>T = 22°C                     | No                    | Also looked at river water degradation                                                       | (Konstantinou et al., 2001) |
| Diuron    | No degradation 42 d     | 1 mg/L        | L = Constant<br>T = 15°C                    | No                    | Included 2 Diuron degradation products: DCPMU ( $T_{1/2}$ = 33 d), DCPMU ( $T_{1/2}$ = 50 d) | (Thomas et al., 2002)       |
| Simazine  | L = 29 d<br>D = 49 d    | 5 mg/L        | L = light (8 h sun)<br>D = dark<br>T = 20°C | No                    | Also looked at river and groundwater degradation and included 2 additional herbicides        | (Navarro et al., 2004a)     |
| Simazine  | S = 29 d<br>T = 28 d    | 5 mg/L        | S = 20°C, light<br>T = 40°C, light          | No                    | Also looked at river and groundwater degradation and included 2 additional herbicides        | (Navarro et al., 2004b)     |
| Simazine  | No degradation for 20 d | 20 µg/L       | L = unknown intensity<br>T = 20°C           | 0.45µm and unfiltered | Noted absence of degradation compounds                                                       | (Meakins et al., 1995)      |

### ***Comparison of herbicide persistence between flask and pond experiments***

The long persistence of the 6 PSII herbicides in the flask experiment under the sterile conditions of the mercuric chloride treatment ( $t_{1/2} > 1000$  days) indicates chemical breakdown by hydrolysis is very limited in seawater. Persistence in the 0.45 µm-filtered water treatments was generally shorter for most herbicides, indicating the limited microbial populations in the absence of particulates are able to contribute to degradation. In the absence of light and added sediments in the ponds, Diuron and Atrazine degraded twice as quickly; indicating metabolism by microbial populations in the ponds was greater. The opposite effect was observed for Hexazinone and Tebuthiuron. This result was not expected as microbial populations were likely to be more diverse and abundant in the ponds. A possible explanation is that the ponds (unfiltered seawater) also contained richer, more available sources of organic carbon that were preferentially consumed by microbial populations. It is also worth noting that correlations for  $\ln$  (concentration) vs time are lower for herbicides with long persistence (Table A2 and A4), therefore direct comparisons between these very long half-life data are less reliable.

## ***Benefits and application***

These results provide researchers, managers, regulators, and industry with reliable persistence data for priority herbicides detected in the GBR lagoon. The long persistence of herbicides in seawater in the pond *Experiment 2*, demonstrates that negligible herbicide degradation is likely in flood plumes lasting for weeks and that herbicides reaching the GBR lagoon may persist there for many months to years. This information has been used in the development of the Risk Assessment process for the Reef Plan Scientific Consensus Statement (Brodie et al., 2013). In addition, these results have been disseminated to a wide range of relevant stakeholders including Department of the Environment (DSEWPaC), DERM, GBRMPA, APVMA, Terrain, SRDC, WWF, Davco Farming, BSES, DAFF, Farmacist, NQ Dry Tropics, Canefarmers, and AgForce, at Implementation Group Meetings, the 2013 NERP-Reef Rescue Conference in Cairns, and at three Pesticide Working Group meetings between 2012 and 2014.

## **Conclusions**

This was the first study to quantify the seawater persistence of priority herbicides detected in the GBR lagoon. Standard Flask *Experiment 1A* revealed extremely slow degradation of Diuron, Atrazine, Hexazinone, Tebuthiuron, Simazine, and Ametryn in the absence of light and sediments, with all herbicides exhibiting half-lives of over 600 days. *Experiment 2* was conducted in outdoor ponds with unfiltered coastal water and treatments included the presence and absence of light and sediments. These conditions more closely mimic coastal habitats in the GBR lagoon than flask experiments in incubators. Under the most environmentally relevant conditions (light + sediments) the order of persistence was Metolachlor (half-life,  $t_{1/2}$  = 32 d) < Atrazine (107 d) < Diuron (139 d) < Hexazinone (201 d) < 2,4-D (288 d) < Tebuthiuron (944 d). The presence of coastal sediments consistently reduced the persistence (1.1 – 6.3-fold) of herbicides, especially in moderate sunlight. Moderate sunlight also reduced the persistence of Diuron, Atrazine, Hexazinone, Tebuthiuron and Metolachlor in the presence of sediments. 2,4-D was a notable exception and was far more persistent in the light. Changes in persistence were most likely due to the influence of light and sediments on microbial populations. The long persistence of herbicides in seawater in the pond *Experiment 2*, demonstrates that negligible herbicide degradation is likely in flood plumes lasting for weeks and that herbicides reaching the GBR lagoon may persist there for many months to years. These results help explain the year-round detection of herbicides in the GBR lagoon.

This reliable persistence data for priority herbicides detected in the GBR lagoon is now available to managers, regulators and industry to enable (in combination with exposure and toxicity data) more effective and targeted management of these agricultural contaminants and develop more accurate spatial risk assessment models for coral reefs and seagrass beds of the GBR. Further work is needed to quantify the fate of dozens of other herbicides, fungicides and insecticides, as well urban and industrial contaminants detected in the GBR lagoon.

## ***Future Directions***

While this project (in combination with NERP 4.2) has examined the persistence of nine priority herbicides in seawater under various tropical marine conditions, there are a large number of insecticides, fungicides and other herbicides registered for use in GBR catchments that are currently not monitored and have not been tested for toxicity or persistence under tropical marine conditions (King et al., 2012). Other pollutants from increasing urban and industrial sources should also be considered. Further work is needed to quantify the fate of these contaminants to ensure the best advice for management and regulators to ensure protection of the GBR from agricultural chemicals in runoff.

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## Appendix 1: Additional Data

**Table A1. Slope of the ln(herbicide concentration) graphs vs time for Flask Experiment 1A. Herbicides were incubated in 0.45 µm filtered water with half the treatments containing mercuric chloride to ensure sterile conditions.**

| Treatment         | Slope of the ln(herbicide concentration) graphs vs time |           |            |             |           |           |
|-------------------|---------------------------------------------------------|-----------|------------|-------------|-----------|-----------|
|                   | Diuron                                                  | Atrazine  | Hexazinone | Tebuthiuron | Simazine  | Ametryn   |
| Filtered water    | -0.000797                                               | -0.000779 | -0.000682  | -0.000751   | -0.001054 | -0.000813 |
| Mercuric chloride | -0.000687                                               | -0.000550 | -0.000667  | -0.000612   | -0.000524 | -0.000568 |

**Table A2.  $r^2$  values of the Slope of the ln(herbicide concentration) graphs vs time for Flask Experiment 1A. Herbicides were incubated in 0.45 µm filtered water with half the treatments containing mercuric chloride to ensure sterile conditions.**

| Treatment         | $r^2$ values of the Slope of the ln(herbicide concentration) graphs vs time |          |            |             |          |         |
|-------------------|-----------------------------------------------------------------------------|----------|------------|-------------|----------|---------|
|                   | Diuron                                                                      | Atrazine | Hexazinone | Tebuthiuron | Simazine | Ametryn |
| Filtered water    | 0.37                                                                        | 0.042    | 0.40       | 0.47        | 0.538    | 0.49    |
| Mercuric chloride | 0.64                                                                        | 0.41     | 0.51       | 0.44        | 0.42     | 0.49    |

**Table A3. Slope of the ln(herbicide concentration) graphs vs time for outdoor Pond Experiment 2. Herbicides were exposed to four combinations of environmental conditions comprising of the presence and absence of light and sediments.**

| Light | Sediment | Slope of the ln(herbicide concentration) graphs vs time |           |            |             |            |             |
|-------|----------|---------------------------------------------------------|-----------|------------|-------------|------------|-------------|
|       |          | Diuron                                                  | Atrazine  | Hexazinone | Tebuthiuron | 2,4-D      | Metolachlor |
| +     | -        | -0.001714                                               | -0.002099 | -0.0005844 | -0.0002081  | -0.0003796 | -0.0073648  |
| +     | +        | -0.004977                                               | -0.006457 | -0.0034533 | -0.0007342  | -0.0024078 | -0.0217938  |
| -     | -        | -0.001388                                               | -0.002313 | -0.0003476 | -0.0003924  | -0.0116596 | -0.0047187  |
| -     | +        | -0.002485                                               | -0.003451 | -0.0006758 | -0.0004701  | -0.0124153 | -0.0067346  |

**Table A4.  $r^2$  values of the Slope of the ln(herbicide concentration) graphs vs time for outdoor Pond Experiment 2. Herbicides were exposed to four combinations of environmental conditions comprising of the presence and absence of light and sediments.**

| Light | Sediment | $r^2$ values of the Slope of the ln(herbicide concentration) graphs vs time |          |            |             |       |             |
|-------|----------|-----------------------------------------------------------------------------|----------|------------|-------------|-------|-------------|
|       |          | Diuron                                                                      | Atrazine | Hexazinone | Tebuthiuron | 2,4-D | Metolachlor |
| +     | -        | 0.944                                                                       | 0.809    | 0.875      | 0.631       | 0.496 | 0.909       |
| +     | +        | 0.960                                                                       | 0.892    | 0.724      | 0.933       | 0.926 | 0.929       |
| -     | -        | 0.870                                                                       | 0.937    | 0.713      | 0.703       | 0.653 | 0.881       |
| -     | +        | 0.973                                                                       | 0.965    | 0.913      | 0.838       | 0.931 | 0.969       |