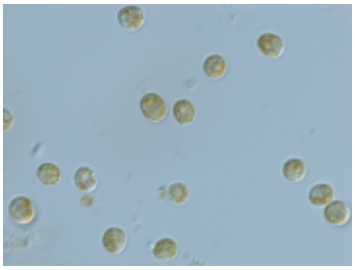
Toxicity of nine herbicides to coral endo-symbiotic algae Cladocopium goreaui (Symbiodiniaceae) (NESP 3.1.5, AIMS and JCU)



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Title	Toxicity of nine herbicides to coral endo-symbiotic algae Cladocopium goreaui (Symbiodiniaceae) (NESP 3.1.5, AIMS and JCU)
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Abstract	This dataset shows the effects of herbicides (detected in the Great Barrier Reef catchments) on the growth rates (from cell density data) and photosynthesis (effective quantum yield) on the free-living form of the dinoflagellate coral symbiont Cladocopium goreaui during laboratory experiments conducted from 2017-2019. The aims of this project were to develop and implement standard ecotoxicology protocols to determine the effects of Photosystem II (PSII) and alternative herbicides on the growth and photosynthetic efficiency of the marine dinoflagellate Cladocopium goreaui. Bioassays were performed over 2-week exposures using herbicides that have been detected in the Great Barrier Reef catchment area (O'Brien et al., 2016). These toxicity data will enable improved assessment of the risks posed by PSII and alternative herbicides to coral endo-symbiotic algae for both regulatory purposes and for comparison with other taxa. Methods: A monoclonal strain of Cladocopium goreaui (formerly Symbiodinium clade C, (LaJeunesse et al., 2018) was isolated from the coral Acropora tenuis near Magnetic Island in Queensland, Australia (Australian Institute of Marine Science strain: SCF 055-01.10). Cultures were maintained in IMK media at 27 ± 1 °C and incubated at 14:10 h light:dark cycles under light intensity of 60-75 µmol m-2 s-1. C. goreaui cultures used for the bioassays were 14 days old and in the logarithmic growth phase. C. goreaui cells were transferred to 50 ml polypropylene tubes with IMK media to a final cell density of 1.7 -2.7 x 104 cells mL-1. Treatments were run with 3-6 replicates, including IMK media controls, solvent controls and a reference toxicant control (6 µg L-1 diuron). C. goreaui cells were exposed for 14 d and temperature maintained at approximately 27°C in a refrigerated incubator shaker. Cells were kept suspended with shaking at 130 rpm. Bioassays were conducted at similar conditions to the mother culture. Herbicide stock solutions were prepared using analytical grade products (Sigma-Aldrich 98-99.5% purity): diuron (CAS 330-54-1), metribuzin (CAS 21087-64-9), hexazinone (CAS 51235-04-2), tebuthiuron (CAS 34014-18-1), bromacil (CAS 314-40-9), propazine (CAS 139-40-2), simazine (122-34-9), imazapic (CAS 104098-48-8), haloxyfop-p-methyl (CAS

72619-32-0). Stock solutions (5 - 600 mg L-1) were prepared in Milli-Q® water or filtered seawater. Diuron and metribuzin were dissolved using the carrier solvent ethanol (final concentration < 0.002% v/v in all exposure treatments). Haloxyfop and simazine were dissolved in the carrier solvent dimethyl sulfoxide (DMSO; final concentration < 0.006% v/v in all exposure treatments). No solvent carrier was used for the preparation of the remaining herbicide stock solutions. Herbicide analysis was done at the Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland using HPLC-MS/MS (SCIEX Triple QuadTM 6500 QTRAP® mass spectrometer Shimadzu Nexera X2 uHPLC sytem) (Mercurio et al., 2015, Mercurio, 2016).

Flow cytometry was used to quantify specific growth rates of C. goreaui using a BD Accuri C6 flow cytometer. Cell densities were determined by plotting a 2-dimensional cytogram with fixed gating. Gating of cells was used to differentiate between C. goreaui and degraded chloroplasts of senescing cells or microbes. Specific growth rate (SGR) was calculated as the logarithmic increase in cell density from day 0 to day 14. SGR relative to the control treatment was used to derive effect values for growth inhibition. Specific growth rates (SGR) were expressed as the logarithmic increase in cell density from day i (ti) to day j (tj) as per equation (1), where SGRi-j is the specific growth rate from time i to j; Xj is the cell density at day j and Xi is the cell density at day i (OECD, 2011).
SGR i-j = [(ln Xj - ln Xi)/(tj - ti)] (day-1) (1)

SGR relative to the control treatment was used to derive effect values for growth inhibition.

Effects of herbicides on the photosynthesis of C. goreaui were measured by chlorophyll fluorescence as the effective quantum yield (Delta F/Fm') using microscopy PAM fluorometry (Walz, Germany) (Schreiber, 1998). Light adapted minimum fluorescence (F) and maximum fluorescence (Fm') were determined and effective quantum yield was calculated for each treatment as per equation (2) (Schreiber et al., 2002). Settings for the bioassays were: actinic light = 1, measuring light = 10-12, damp = 2, gain = 3, actinic light width = 180 seconds and saturation pulse intensity = 2.

Delta F/Fm’ = (Fm’-F)/Fm’ (2)

Delta F/Fm' was measured after 13-15 d exposure. Percent inhibition was calculated relative to controls according to (3)

% inhibition = [(Xcontrol – Xtreatment) /Xcontrol] x 100 (3)

where Xcontrol is the mean SGR (or Delta F/Fm') of control and Xtreatment is the mean SGR or (Delta F/Fm') of treatments.

Format:
Cladocopium goreaui herbicide toxicity data_final_eAtlas.xlsx

Data Dictionary:

There are two tabs for each herbicide in the spreadsheet. The first of each pair is the specific growth rate (SGR) data, while the second of each pair is the pulse amplitude modulation (PAM) fluorometry (Delta F/Fm’ data.
The last tab in the spreadsheet is the measured water quality (WQ) data for each herbicide bioassay.

Diu – Diuron
Brom – Bromacil
Halo – Haloxyfop
Hexa – Hexazinone
Imaz – Imazapic
Met – Metribuzin
Prop – Propazine
Sim – Simazine
Teb – Tebuthiuron

For each ‘herbicide’_SGR tab:
SGR = specific growth rate – the logarithmic increase from day 0 to day 14

Nominal (µg/L) = nominal herbicide concentrations used in the bioassays; SC denotes solvent control which is no herbicide and contains less than 0.006% v/v solvent carrier as per the treatments; D6 denotes diuron reference at 6 µg/L
Measured (µg/L) = measured concentrations analysed by The University of Queensland; Diuron reference toxicant at 6 µg/L was not measured
Rep = Replicate: for SGR, notation is 1-10; for PAM data, notation is A, B, C
T14_CellsPerMl = cell density at day 14 (except for simazine in which day 10 was used – notation 'T10_CellsPerMl')
ln(Day 14) = natural logarithm of cell density at day 14 (except for simazine in which day 10 was used – notation 'ln(Day 10)')
T0_CellsPerMl = cell density at day 0
ln(Day 0) = natural logarithm of cell density at day 0

For each 'herbicide'_PAM tab.
PAM = pulse amplitude modulated fluorometry to calculate effective quantum yield (light adapted)
Nominal (µg/L) = nominal herbicide concentrations used in the bioassays; SC denotes solvent control which is no herbicide and contains less than 0.006% v/v solvent carrier as per the treatments; D6 denotes diuron reference at 6 µg/L
Measured (µg/L) = measured concentrations analysed by The University of Queensland; Diuron reference toxicant at 6 µg/L was not measured
Rep = Replicate: for SGR, notation is 1-10; for PAM data, notation is A, B, C
No = individual C. goreaui cell used to measure effective quantum yield
Delta F/Fm' ' = effective quantum (light adapted) yield measured by a microscopy Pulse Amplitude Modulated (microscopyPAM) fluorometer

'WQ' tab:
WQ = water quality
DO = dissolved oxygen measured in mg/L and in % saturation
Imazapic concentrations highlighted in red were removed from calculating mean WQ parameters since the pH at those concentrations were > 1.5 units from the control

References:

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Data Location:

This dataset is filed in the eAtlas enduring data repository at: data\nesp3\3.1.5_Pesticide-guidelines-GBR

Metadata language	eng
Character set	UTF8
Hierarchy level	Dataset

OnLine resource

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Protocol	WWW:LINK-1.0-http--related
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Linkage	https://eatlas.org.au/pydio/public/au-nesp-twq-3-1-5-aims-pesticide-guidelines-gbr-cladocopium-goreaui-2020-05-07
Protocol	WWW:LINK-1.0-http--downloaddata

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Extent

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