Photochemical production of oxylipin infochemicals in West Antarctica: An approach using new tools for semi-untargeted lipidomics

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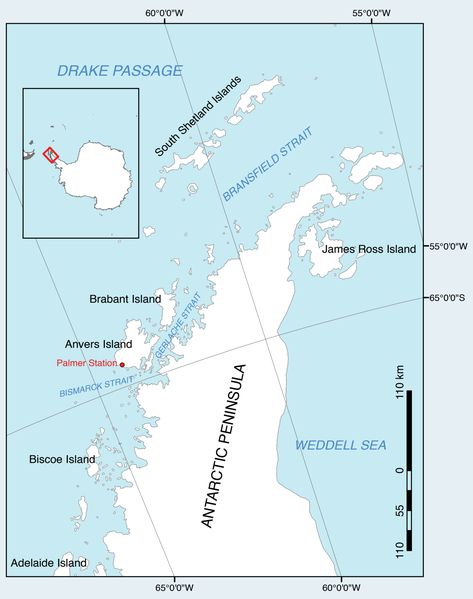
Introduction

We combine **high-resolution mass spectrometry** & **semi-untargeted lipidomics** to observe simultaneous changes over time in a **broad suite of compounds** involved in the **photochemical production** of **oxylipins** in the **suface ocean**

Oxylipins are bioactive **"infochemicals"** produced by **oxidation of intact lipids**. Oxylipins have **antimicrobial properties** and act as **stress/defense signals** in many terrestrial plant and aquatic systems. They can be produced by:

- regulated, **biologically-mediated pathways** (e.g., lipoxygenase)
- **abiotic** sources of **reactive oxygen species**, such as **photooxidation** (the subject of this study)

Study locations



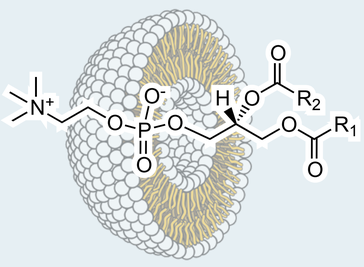
The **West Antarctic Peninsula** (WAP) receives **abnormally high doses of ultraviolet-B (UVB) radiation** as a result of stratospheric ozone depletion

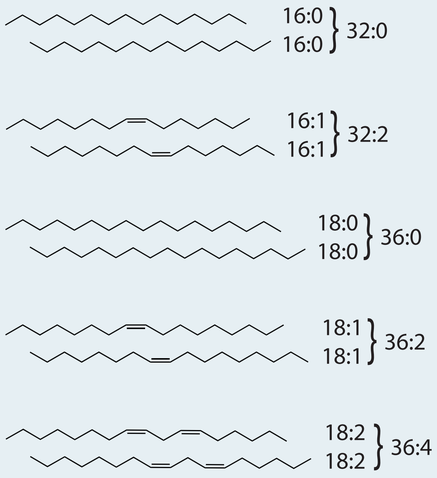
This **UVB** flux **increases surface concentrations of reactive oxygen** in austral spring

At the U.S. **Palmer Station**, we conducted a series of **time-course lipid degradation experiments** with **liposomes** under the **natural light field**

Environmental samples: Also collected at Palmer Station & at several coastal stations

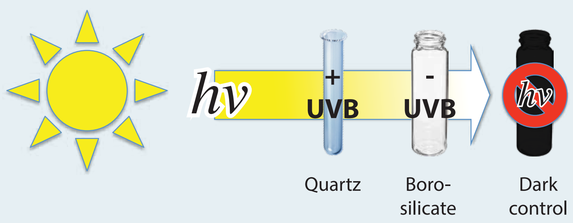
Photodegradation experiments





Liposomes of various moities of **phosphatidylcholine (PC)**, a phospolipid abundant in **diatom cell & thylakoid membranes**, were subjected to **three light treatments**; unsaturated PC species were either Δ9-*cis* or 9,12-*cis*

Liposomes were added to glass vials in a **matrix** of 0.2 µm **filtered natural seawater** — containing both **DOC** and **NO_3^-** as potential chromophores — and **incubated at 1 m depth** in large-volume **on-deck aquaria**



x 3 replicates/ treatment

x 5 timepoints

Sample collection & HPLC/ESI-MS method

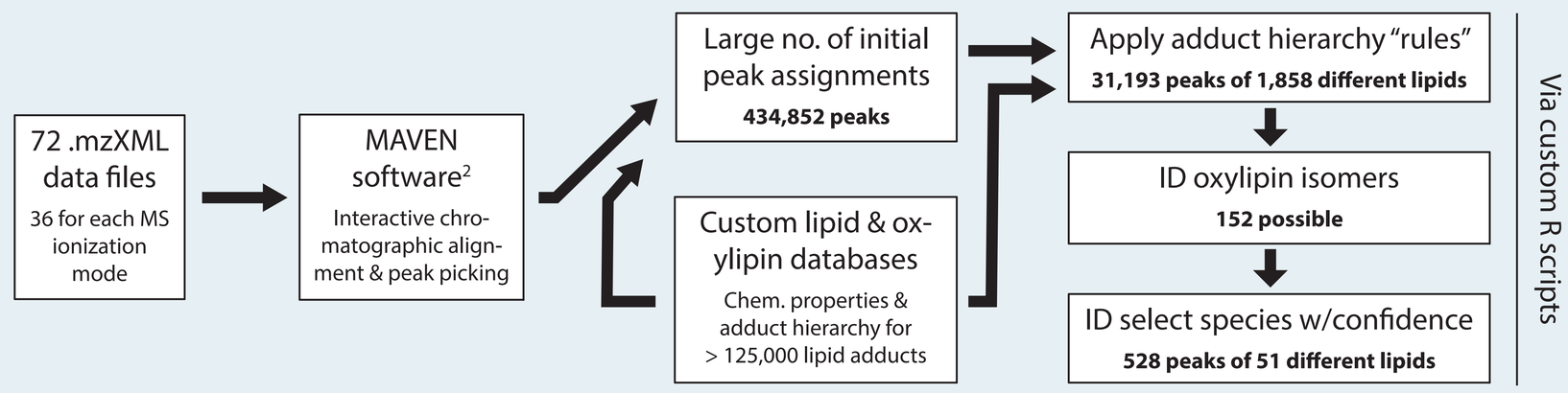
After incubation (experiments) **or retrieval via CTD** (environmental samples), lipids & oxylipins were extracted in 9:1 DCM : MeOH; an **internal standard** was added

HPLC/ESI-MS analysis: Thermo Exactive Orbitrap with reversed-phase chromatography; 100-1500 *m/z* scan range w/ionization mode switching (adapted from ref. 1)

For each experiment and environmental data set, **sample data were then pooled** and **screening** was performed using the **lipidomics data analysis pipeline** described in the adjacent column

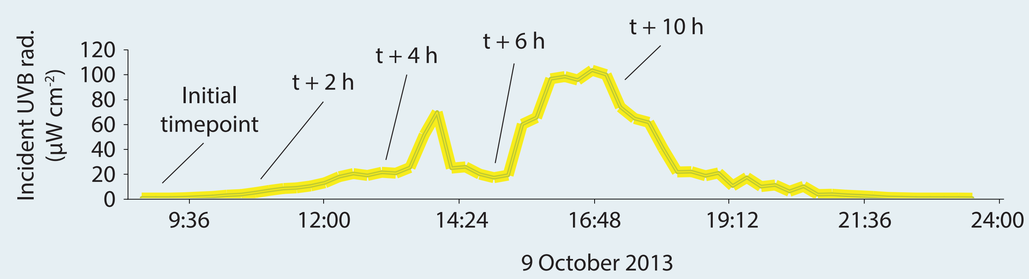
[1] Hummel et al. 2011, *Front. Plant Sci.* 2. [2] Melamud et al. 2010, *Anal. Chem.* 82 (23): 9818-9826. [3] Lipidomics analysis metrics reported for positive mode HPLC/ESI-MS data (36 samples) from the Oct. 9, 2013, experiment. [4] Asterisk denotes significance of treatment difference from control (ANOVA; $p < 0.05$); error bars: ± SE. [5] Structures putative; determination of specific sites of oxidation is part of planned future analysis.

Lipidomics data analysis pipeline[3]



Results from a lipid photodegradation experiment

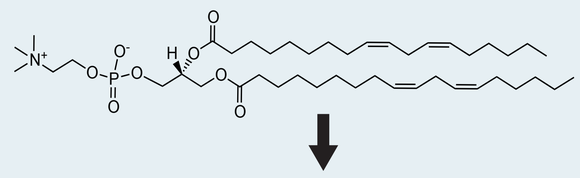
Incident **UVB radiation** (315-290 nm) during experiment and **sampling timepoints**



Evidence in **differences between treatments**[4] for photooxidation of an intact parent lipid, production of oxidized intermediate, and accumulation of a "terminal" oxylipin

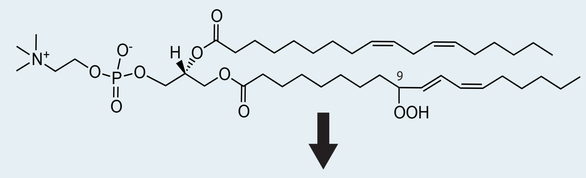
Loss of unoxidized parent compound

18:2, 18:2 (Δ9,12-*cis*) PC (36:4 PC)



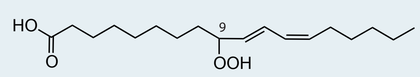
Production of intact intermediate

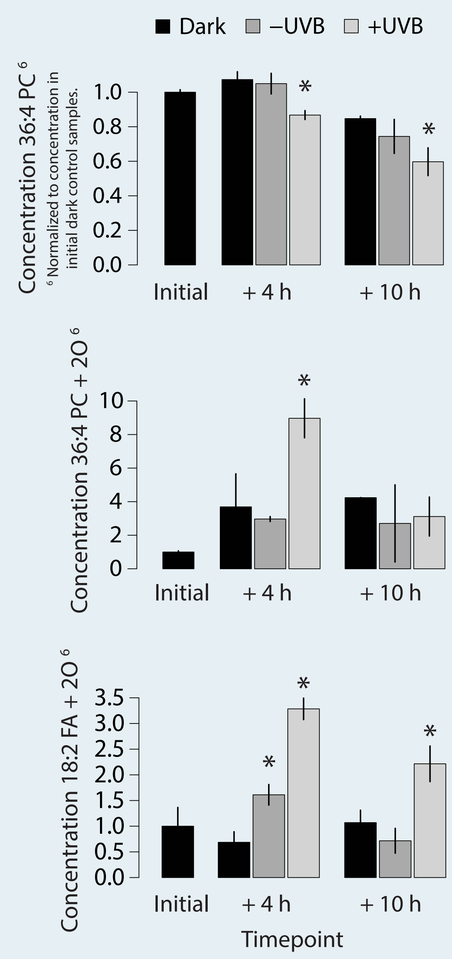
36:4 PC + 2O hydroperoxide[5]



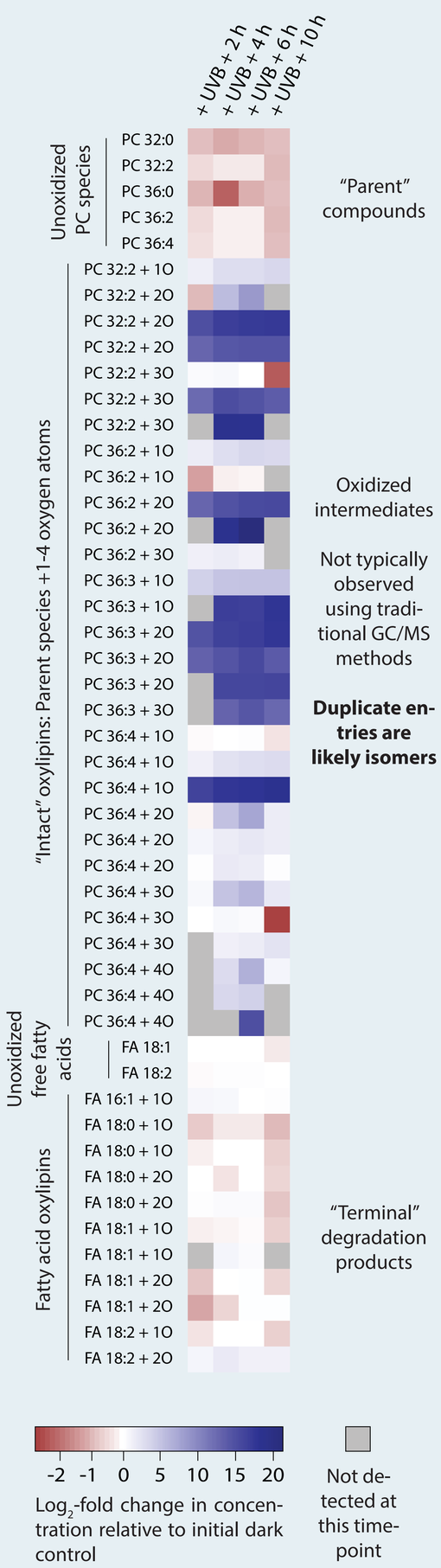
Accumulation of degradation product

18:2 + 2O hydroperoxy (oxylipin) fatty acid[5]





Insight from **lipidomics**: Tracking the **fates of multiple species** over time



Summary of results & significance

With **high-resolution mass spectrometry** and a new **lipidomics pipeline,** we:
- **quantify thousands of compounds** at multiple positions in the lipid photooxidation reaction chain, and
- **identify**, with high confidence, **~ 10^3 oxylipins** in a typical data set

Whereas rates of photooxidation are typically quantified indirectly (via assays such as TBARS or MDA) or by measuring only the accumulation of terminal degradation products (e.g., by GC/MS), our method allows us to **quantify rates from both upstream & downstream of a biogeochemically significant process**

Acknowledgements

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