The environmental effects of diluted bitumen on eelgrass (Zostera marina)

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The Effects of Diluted Bitumen on Marine Intertidal Vascular Plants

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Kinder Morgan Trans Mountain Pipeline Expansion

- Addition of approx. 987 km of pipeline
- Construction of 3 new marine berths at Westbridge Marine Terminal
- New pipeline designed to carry heavier oils: diluted bitumen (Dilbit)
- System capacity increase from 300,000 to 890,000 barrels/day
- Increase in oil tanker traffic in Burrard Inlet from approx. 5 to 37 per month
- Shipment of dilbit to oversea markets for processing poses risk of a marine spill
Diluted Bitumen

- Dilbit: bitumen diluted with natural gas condensate

- Dilbit is a complex mixture of hydrocarbons including LMW saturates, mono and di-aromatics

- Fate and behavior of spilled dilbit depends on weathering

- Dilbit floats in water but may sink after weathering of lighter components

- Weathered dilbit is known to adhere to suspended sediments in water column

- Little information exists on toxic effects of dilbit on intertidal vascular plants

https://www.wisconsinwatch.org/2014/03/spill-response-inadequate-for-tar-sands-crude-on-great-lakes/
Species Selection

- Study species: eelgrass (Zostera marina)

- Eelgrass meadows fulfill a wide range of ecosystem functions

- Approx. 80% of commercially significant fish and shellfish species use seagrass beds

http://www.seadocsociety.org/species/plants/grasses/eelgrass-zostera-marina/
### Previous Studies

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Field or Lab</th>
<th>Event</th>
<th>Oil &amp;/or Dispersant</th>
<th>Effect</th>
<th>Effect Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>den Hartog and Jacobs (1980)</td>
<td>F</td>
<td>Amoco Cadiz, France</td>
<td>crude &amp; fuel oil</td>
<td>“Not harmed at all”</td>
<td>N</td>
</tr>
<tr>
<td>Jacobs (1980)</td>
<td>F</td>
<td>Amoco Cadiz, France</td>
<td>crude &amp; fuel oil</td>
<td>“Temporary phenomenon”</td>
<td>T</td>
</tr>
<tr>
<td>Dean et al. (1998)</td>
<td>F</td>
<td>Exxon Valdez, Alaska</td>
<td>Prudhoe Bay crude oil</td>
<td>“Injuries to eelgrass, appeared to be slight, did not persist for more than a year after spill.”</td>
<td>T</td>
</tr>
<tr>
<td>Scarlett et al. (2005)</td>
<td>L</td>
<td>-</td>
<td>Superdispersant 25, Corexit 9527</td>
<td>“dispersants disrupted the Photosystem II (PSII) apparatus”</td>
<td>Y</td>
</tr>
<tr>
<td>Cosco Busan Oil Spill Trustees (2012)</td>
<td>F</td>
<td>California</td>
<td>IFO-380 Heavy fuel oil (Bunker)</td>
<td>“there is little evidence to suggest serious injuries to many eelgrass beds were exposed to oil”</td>
<td>N</td>
</tr>
</tbody>
</table>
Experimental Objective

- Objective: “To determine the lethal and sublethal toxicity of environmentally relevant concentrations of dilbit to Pacific marine vascular plant species.”

- Completion of an acute 1 month bioassay to determine lethal effects of dilbit on Zostera marina

- Measurement of several biological endpoints to determine sublethal effects of dilbit on Zostera marina including:
  - Plant growth
  - Chlorophyll $a$ content
  - Chlorophyll fluorescence
  - Stress management pathways

http://northernbushcraft.com/seaweed/eelGrass/notes.htm
Eelgrass Collection and Processing

- Shoots were collected in late July from Boundary Bay, a local uncontaminated reference area.

- Harvesting of shoots followed standardized protocol. Sediment also harvested from site.

- Shoots were processed within 12 hours of collection following standard transplanting protocol.

- All shoots underwent a two week acclimation period prior to treatment

https://en.wikipedia.org/wiki/Boundary_Bay
Treatment

- Water-accommodated fraction (WAF) of dilbit prepared using a glass carboy system designed by the Kennedy Lab.

- WAF contains dissolved components of dilbit, typically LMW hydrocarbons

- The system: stainless steel stirring rods powered by a 12VDC gearmotor rotating at 168 RPM.

- 5 mL of dilbit mixed with 23 L of seawater for 18 hours followed by a 1 hr rest period to achieve the max WAF.

- WAF mixed with seawater to generate five exposure concentrations following logarithmic decline: 100%, 32%, 10%, 3.2%, 1%
Procedure: Long-Term Exposure

- Shoots were exposed to 5 concentrations of Dilbit WAF: 100%, 32%, 10%, 3.2%, 1% and negative control
- Chlorophyll \(a\) content measured every 8 days
- At termination of exposure, shoots were photographed and the following end points were measured:
  - effective quantum yield of Photosystem II (\(\Delta F/Fm'\)) and electron transfer rate (ETR)
  - Chlorophyll \(a\) content
  - Growth
Procedure: Short-Term Exposure

- Shoots were exposed to 3 concentrations of Dilbit WAF: 100%, 32%, 3.2% and negative control
- Shoots were destructively sampled on days: 0, 1, 2, 3, 4 and 9
- ROS Analysis completed immediately remainder of blade and rhizome tissue frozen at -80°C
- Samples preserved for analysis of: catalase activity; SOD activity, protein oxidation
Preliminary Results

No Deaths!
Preliminary Results: Long-term

Chlorophyll $a$ Concentration
Preliminary Results: Short-term

Total ROS in Rhizomes

Total ROS in Blades
Proposed Follow up Studies

- Based on lessons learned through this project, inconsistencies in the literature and the critical role of *Zostera marina* in coastal ecosystems, further studies are recommended. Possible studies include:
  - Long term exposure measuring growth via % increase in rhizome internodes;
  - Short term exposure measuring ROS over initial 24 hours of exposure;
  - Exposures at higher temperatures reflective of local temperature increases resulting from climate change;
  - Germination exposure.
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