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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

http://www.vancouversun.com/business/Photos+Kinder+Morgan+Westridge+Marine+Terminal+Burnaby/11112423/story.html

https://get2yes.icba.ca/projects/trans-mountain-pipeline

http://www.ceaa-acee.gc.ca/050/details-eng.cfm?evaluation=80061

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

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

- 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

Previous Studies

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

Fonseca et al. (2017)

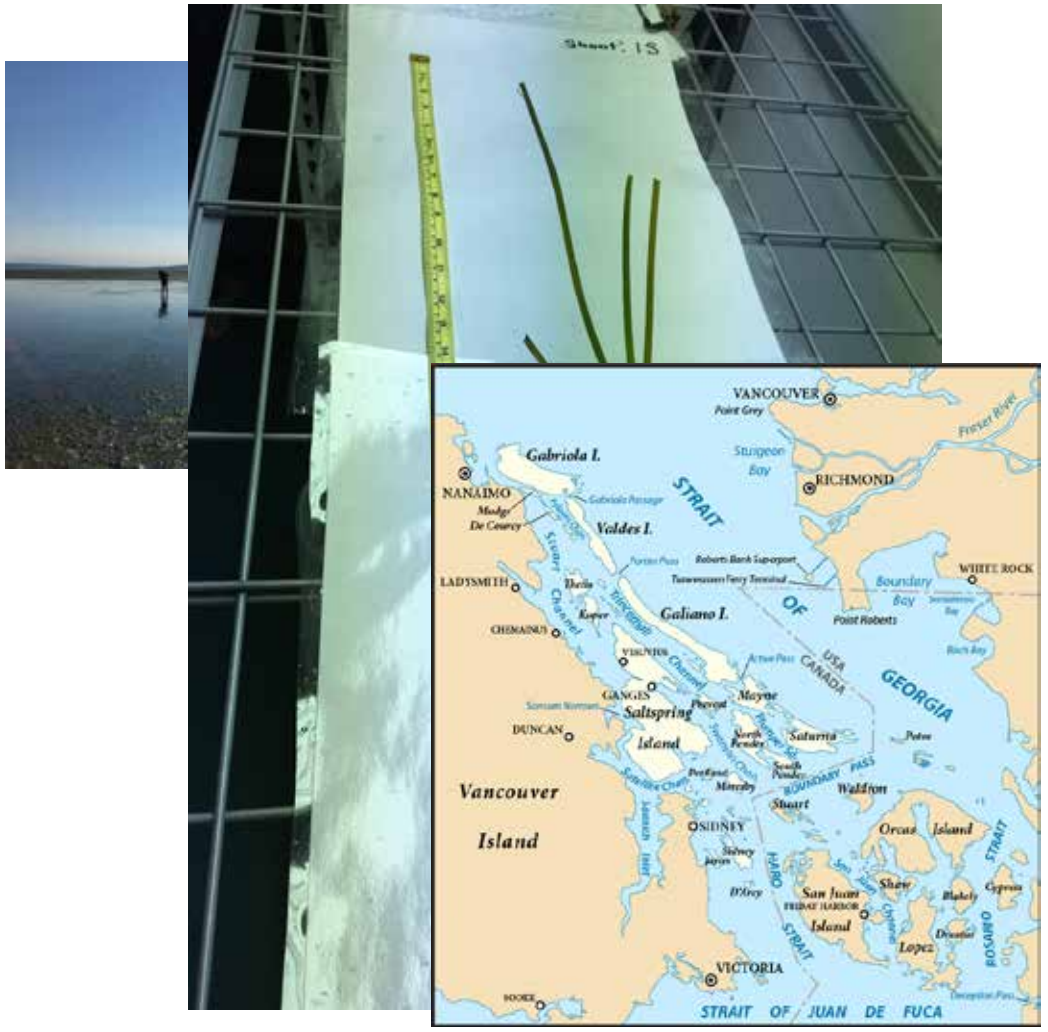
Experimental Objective

- 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

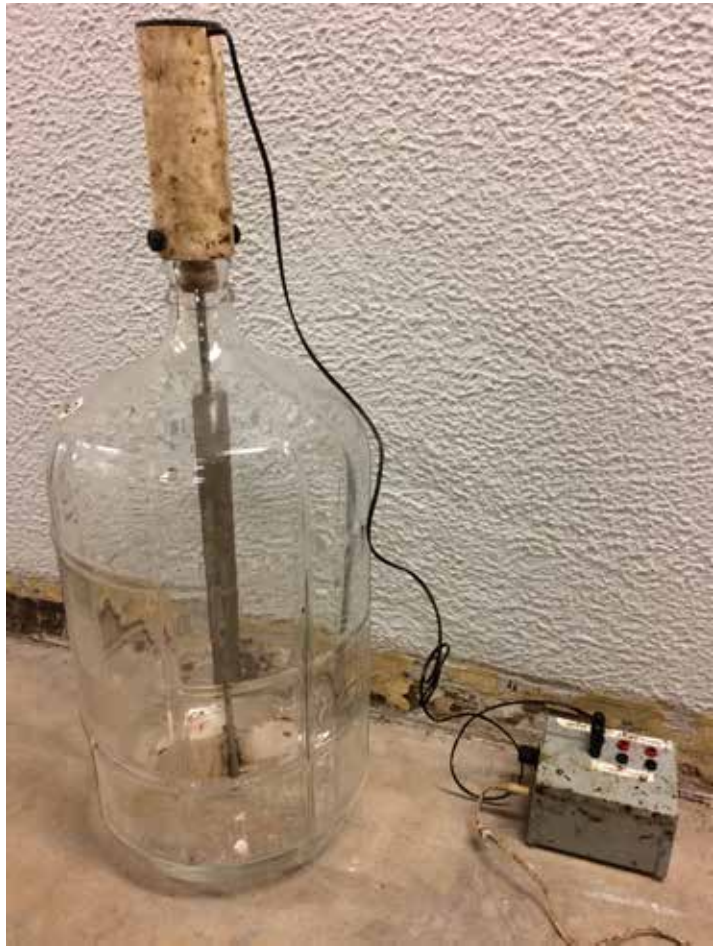
- Shoots were collected in late July from Boundary Bay, a local uncontaminated reference area.
- Harvesting of shoots followed standardized protocol. Sediment also harvested from site.
- Shoots were processed within 12 hours of collection following standard transplanting protocol.
- All shoots underwent a two week acclimation period prior to treatment



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

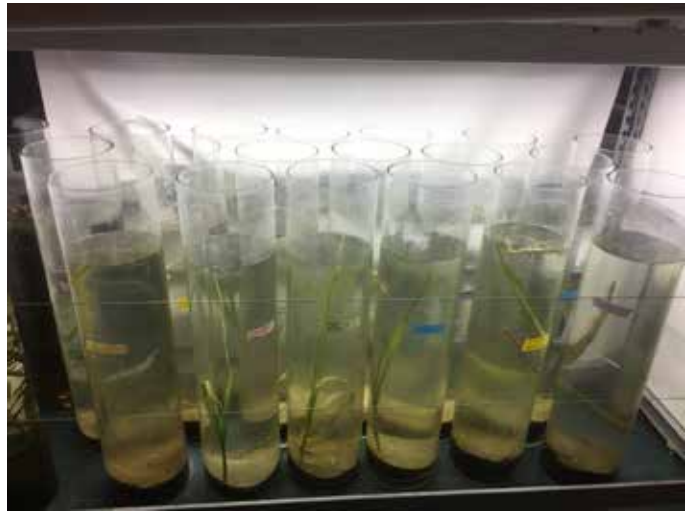
Treatment

- 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 (ΔF/Fm') and electron transfer rate (ETR)
  - Chlorophyll *a* content
  - Growth



Procedure: Short-Term Exposure

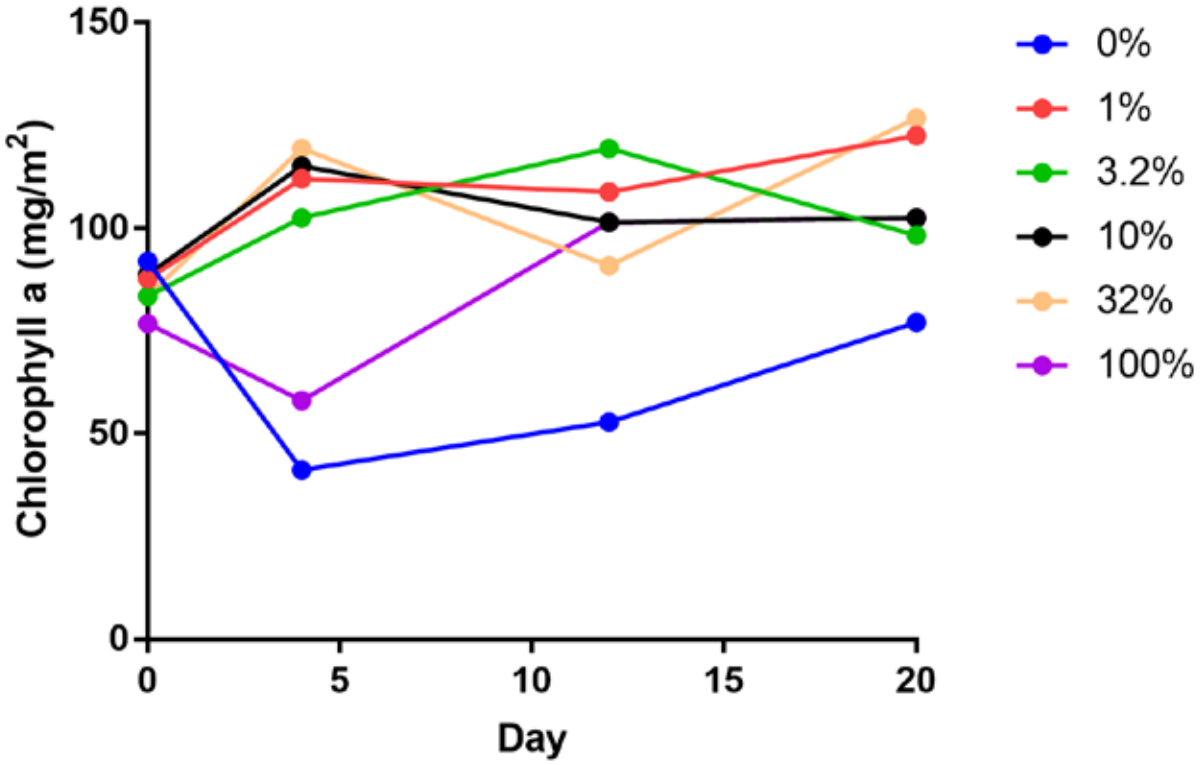
- Shoots were exposed to 3 concentrations of Dilbit WAF: 100%, 32%, 3.2% and negative control
- Shoots were destructively sampled on days: 0, 1, 2, 3, 4 and 9
- ROS Analysis completed immediately remainder of blade and rhizome tissue frozen at -80°C
- Samples preserved for analysis of: catalase activity;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

Preliminary Results

No Deaths!

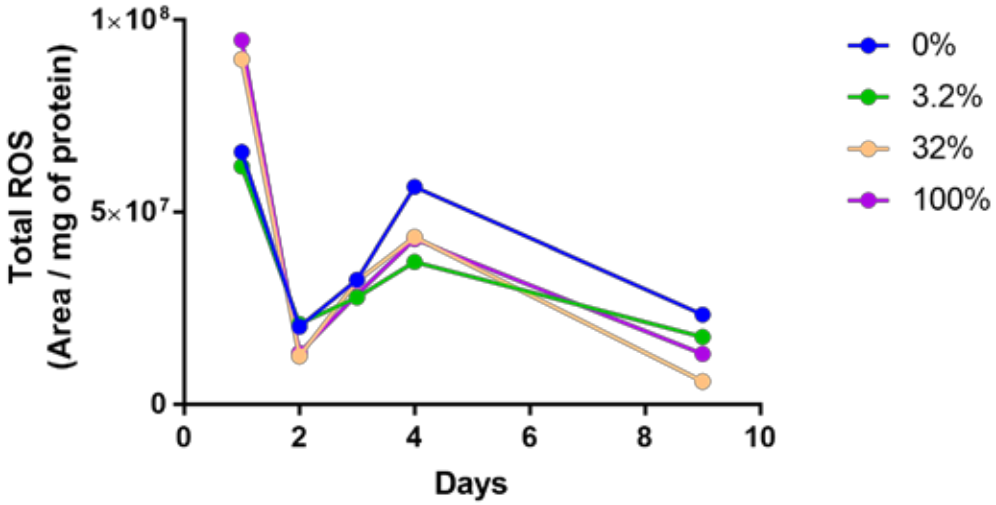
Preliminary Results: Long-term

Chlorophyll *a* Concentration

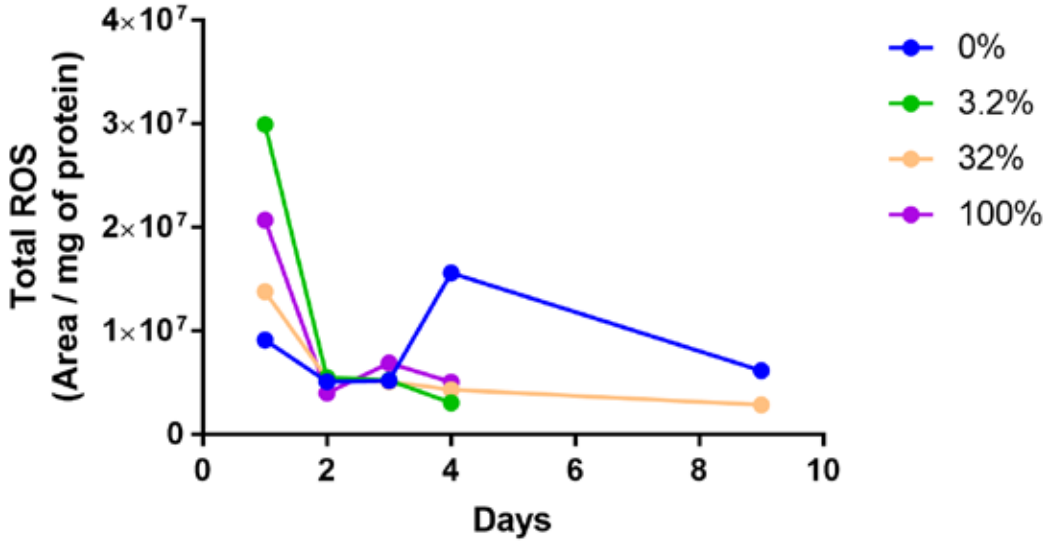


Preliminary Results: Short-term

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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