

Effects of oil and dispersants on *Swiftia exserta*, a structure-forming deep-water octocoral from mesophotic reefs in the Gulf of Mexico

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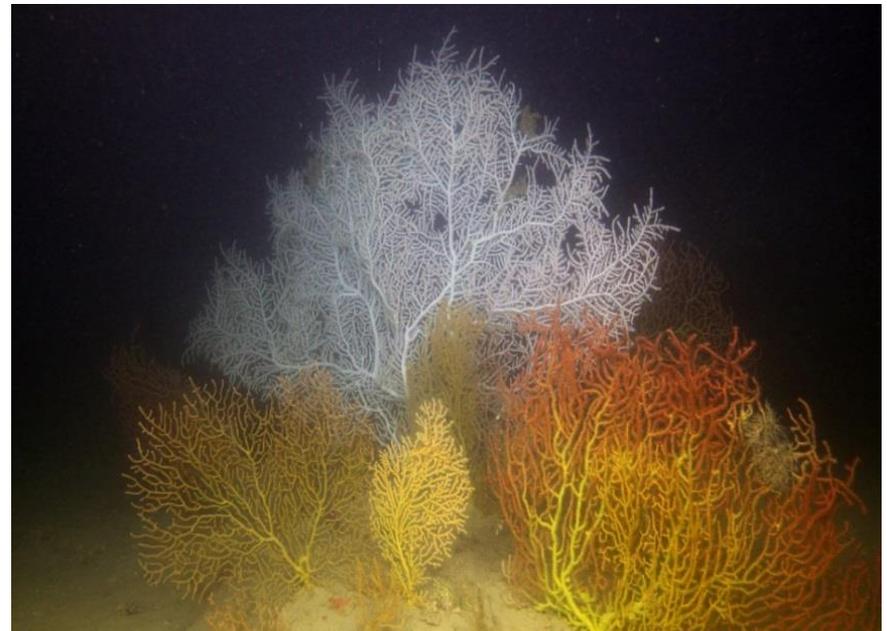
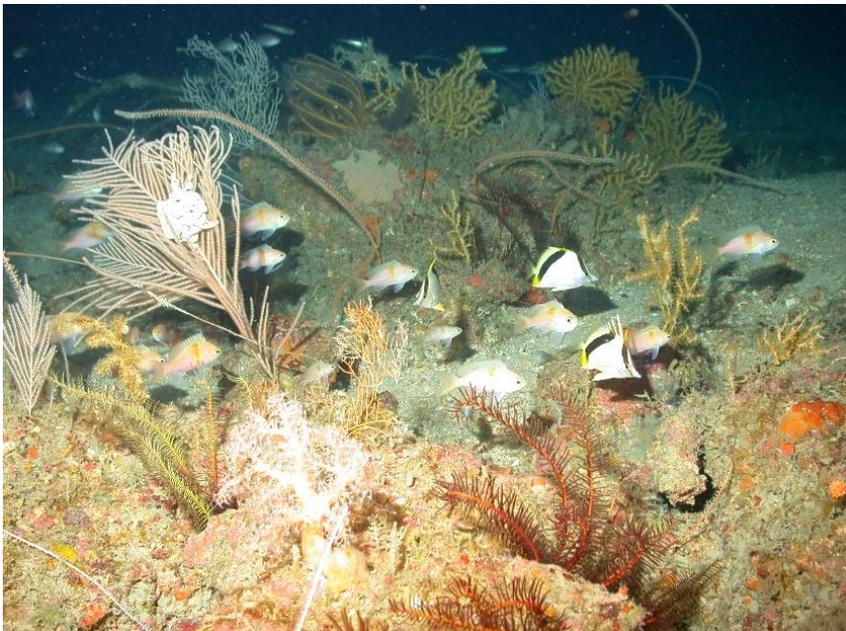
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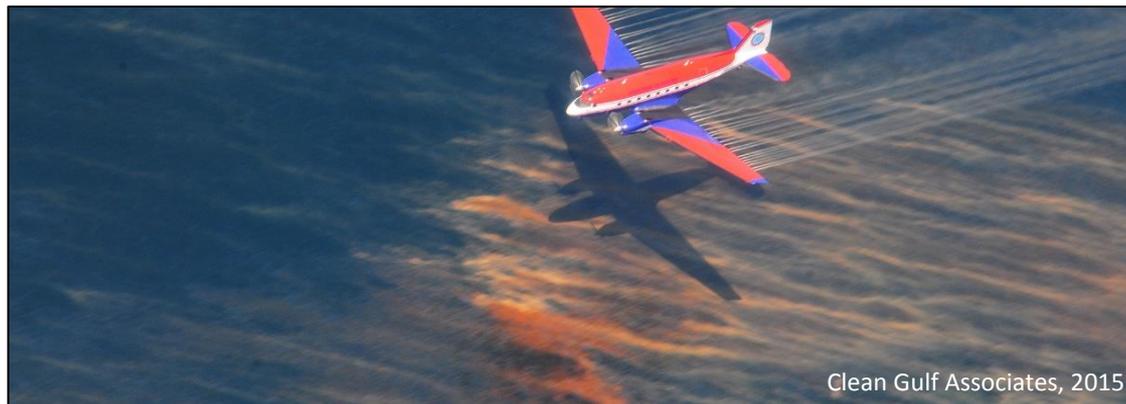
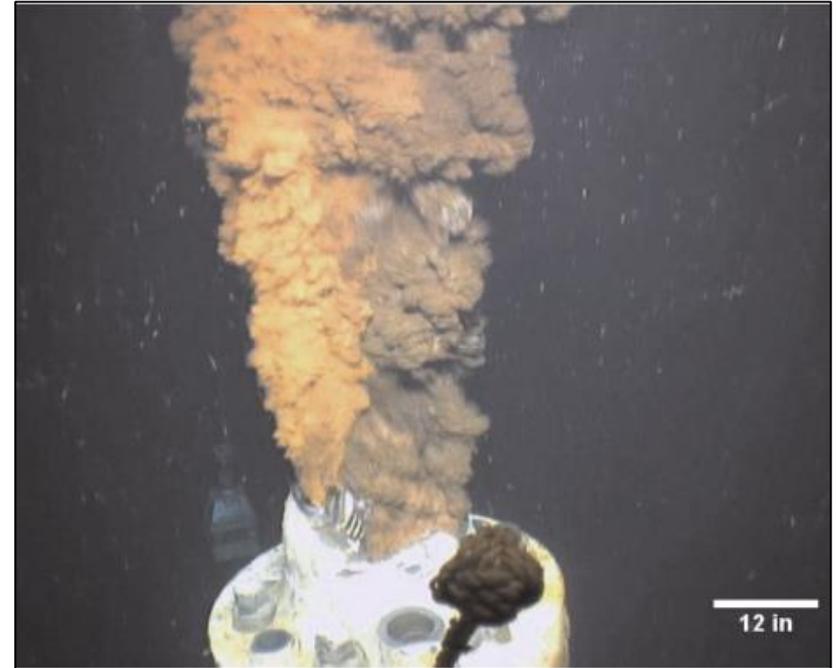


Mesophotic Reefs

- Hard-bottom features
- Occur at 30-150 meters
- Diverse ecosystems of invertebrates and fish
- Heterotrophic corals, vulnerable to pollution
- Sedimentation, fishing impacts, predation, oil and gas exploration



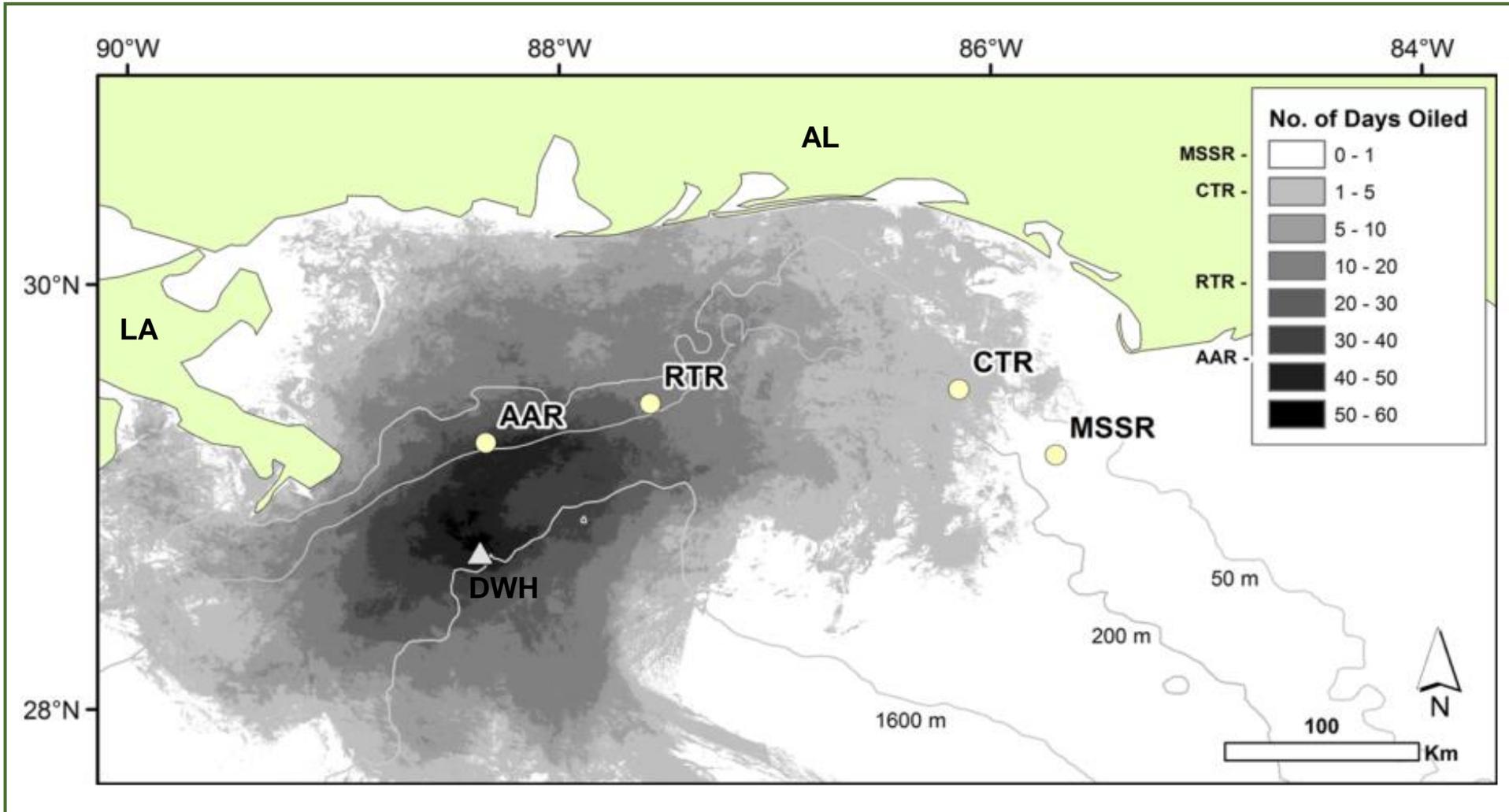
Deepwater Horizon Oil Spill



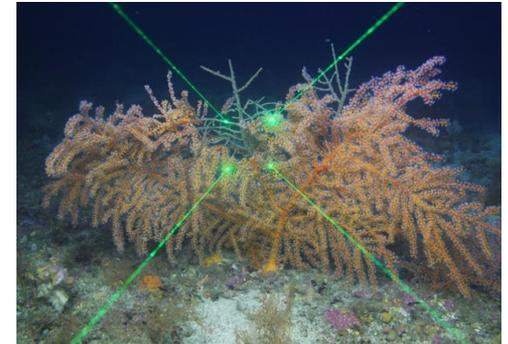
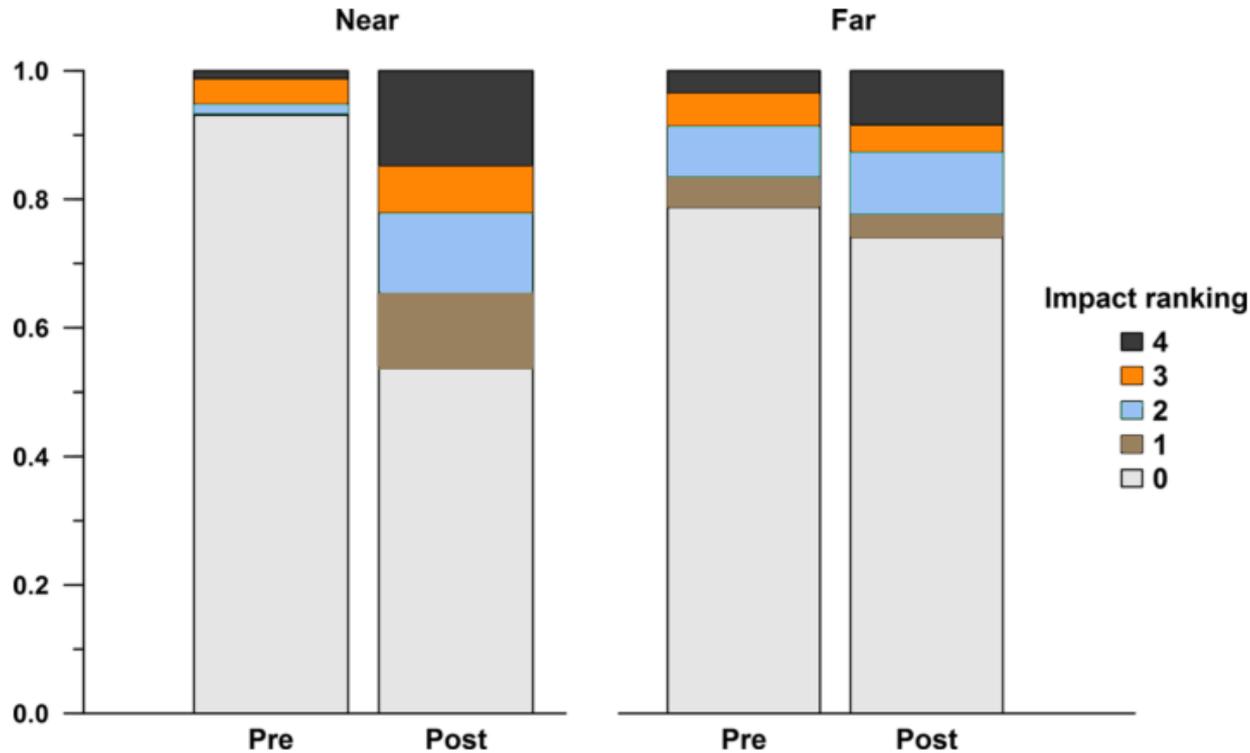
NOAA, 2015

Clean Gulf Associates, 2015

Deepwater Horizon Oil Spill



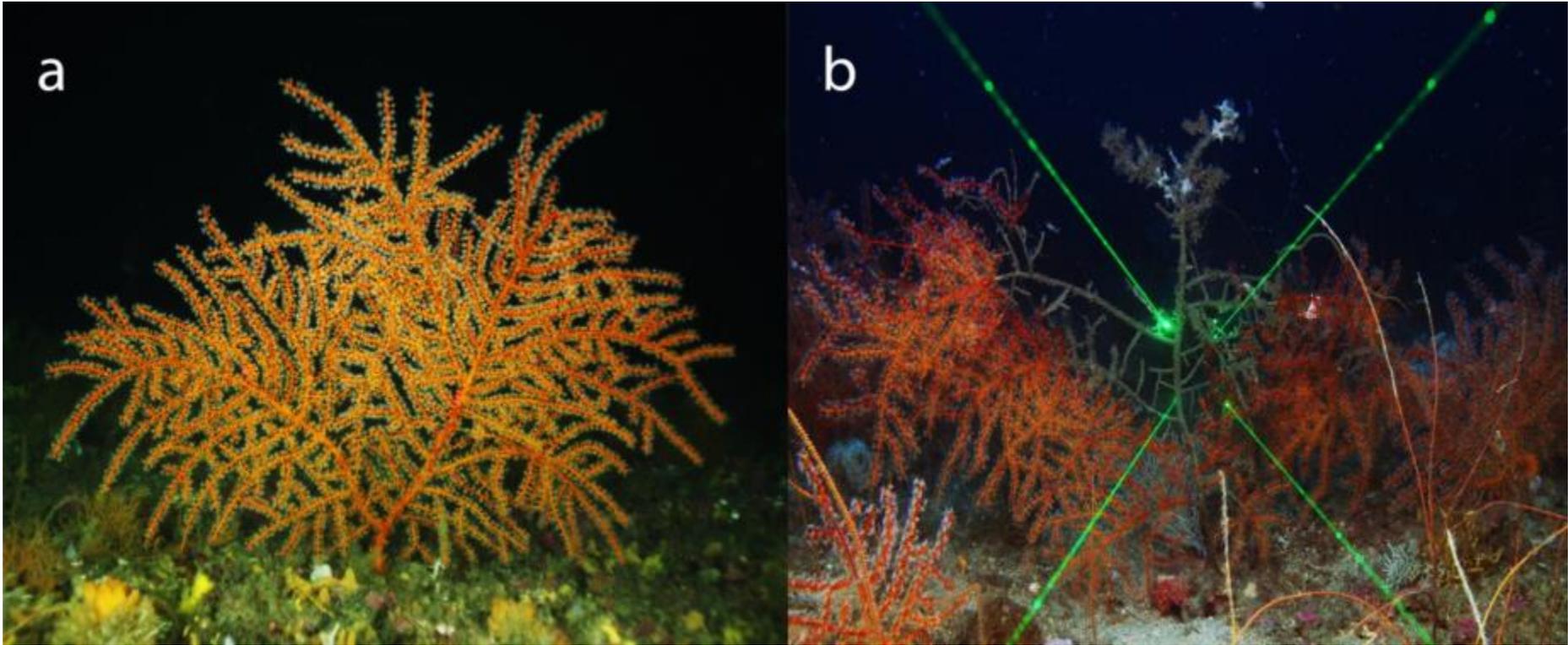
Impact to corals



System wide impacts- water column, benthos, fish

- Massive decline in abundance of demersal reef fish (Sulak & Dixon, 2015)
- Evidence of PAHs in coral tissues (up to 345 ppb) and sediment (up to 101 ppb), but concentrations were low (Silva et al., 2016)
- DWH oil present in sediment traps and semi-permeable membrane devices (NOAA PDARP, 2015)

Study Animal, *Swiftia exserta*



Etnoyer et al., 2016

- Octocoral species occurs throughout West Atlantic, 10-200 m
- Available from Florida through the aquarium trade
- 40-50% *Swiftia* sea fans at study sites injured post-spill

Two morphotypes of *S. exserta*



Swiftia exserta
Southeastern Florida
Red/orange polyps

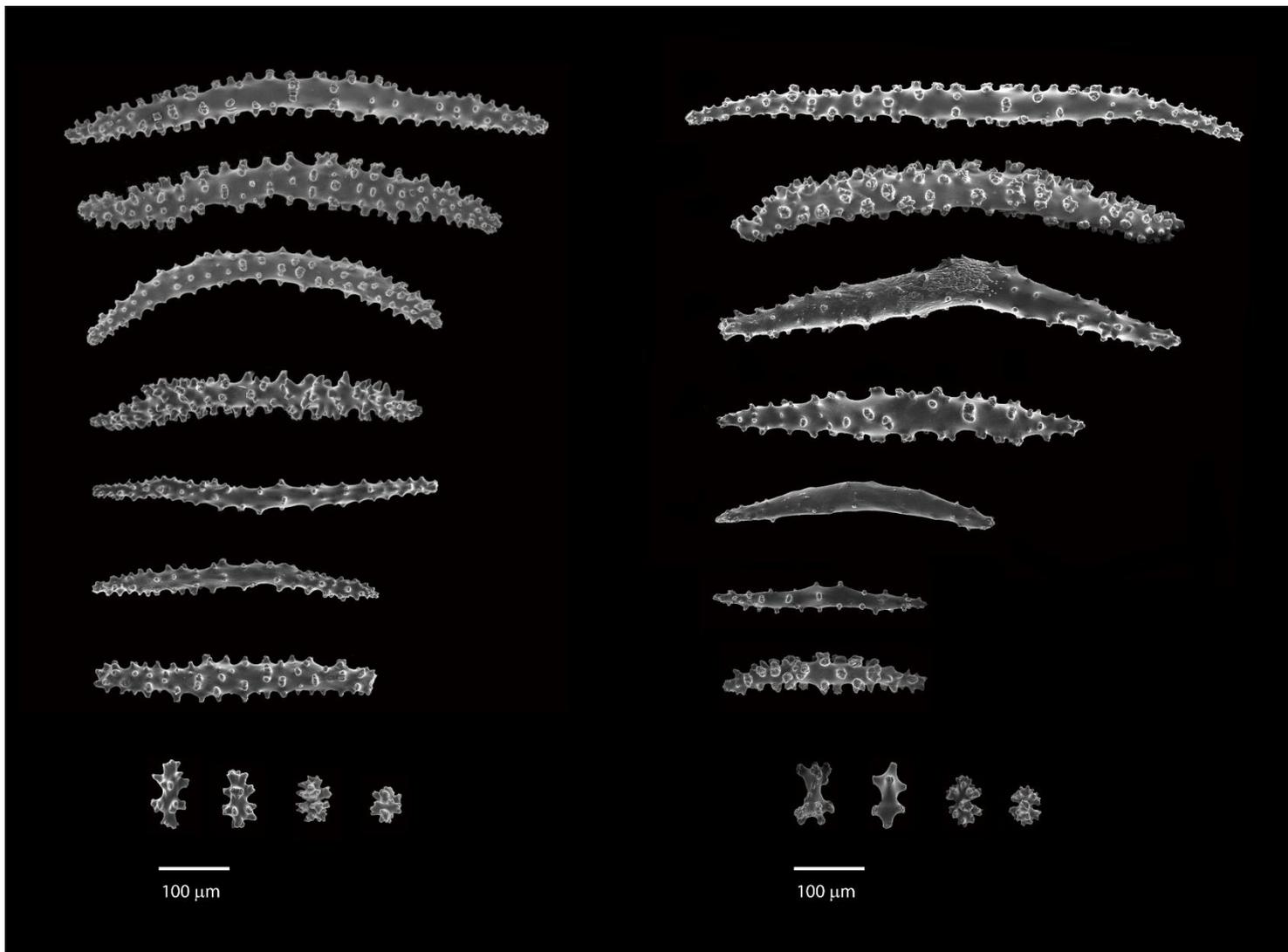


Swiftia sp.
Northern Gulf of Mexico
White polyps

Research Objectives

- I. Resolve the identity of the new phenotype of *Swiftia* by comparing sclerite morphology and DNA sequences of mitochondrial gene *mutS* to *S. exserta*
 - Scanning electron microscopy (SEM)
 - PCR amplification

- II. Examine the effects of Louisiana Sweet crude oil and Corexit 9500©, individually and in mixture, on live *Swiftia exserta*
 - How does *Swiftia* respond to oil and/or dispersant exposure?
 - What is its toxicity threshold when exposed to oil and/or dispersant?



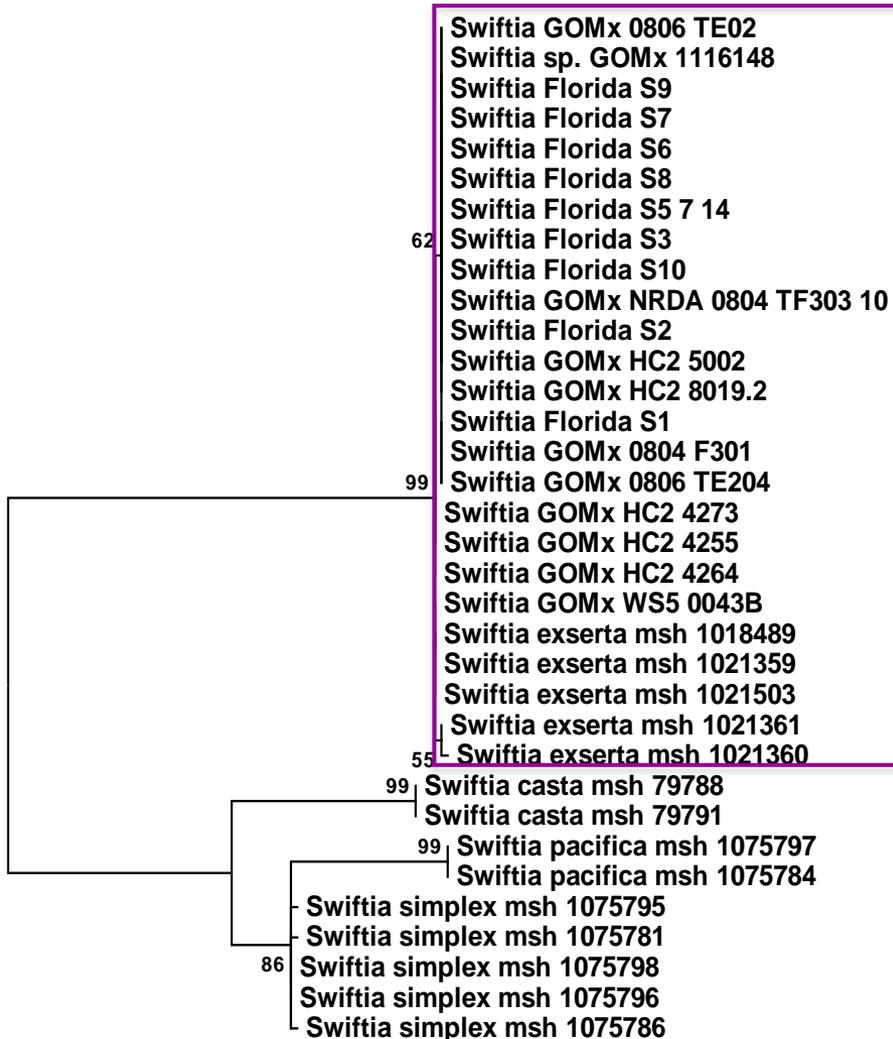
Swiftia exserta
Southeastern Florida
Red/orange polyps

Swiftia sp.
N. Gulf of Mexico
White polyps

Molecular Barcoding

- Amplified and sequenced mtMutS gene region
 - 10 *Swiftia* sp. from Northern Gulf of Mexico
 - 9 *S. exserta* from Southeastern Florida
- 584 base pairs
- Maximum Likelihood
- Tamura-Nei model w/ gamma
- Inc. sequences from Smithsonian NMNH (H Wirshing)
 - 5 *Swiftia exserta* from Caribbean, plus other *Swiftia* spp.

Molecular Barcoding



0.02

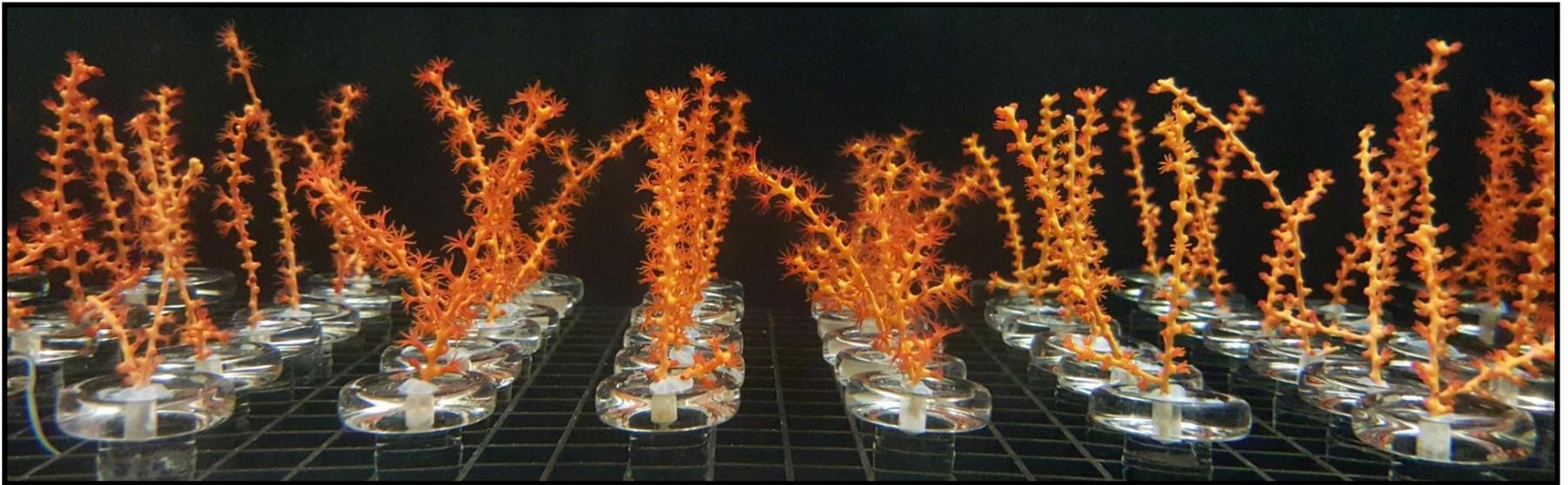
Region/Haplotype	A	B	C	D	Total
Northern Gulf of Mexico	7	4	-	-	11
Florida	9	-	-	-	9
Caribbean	-	3	1	1	5
Total	16	7	1	1	25

No significant difference among *S. exserta*

Regional variability is evident within this species

Experimental Methodology

Short-term (96-hr) toxicological assays of *Swiftia exserta* fragments



Louisiana sweet crude oil water-accommodated oil fractions (WAFs)

Corexit 9500 dispersant

Chemically-enhanced WAFs (CEWAFs, 20:1 oil:Corexit)

Preparation of Treatments



DeLorenzo et al., *in prep*; Hemmer et al. 2011

**Added 25 g/L Louisiana Sweet Crude Oil in filtered seawater
1.25 g/L Corexit (CEWAF)
Stir for 18 hours, Sit for 6 hours
Collect WAF/CEWAF; Leave oil slick behind
Make serial dilutions from full-strength (100%) WAF/CEWAF**

Experimental Methodology

Design

5 experimental groups and 1 control
4 replicates (coral frags) per group

Exposure

Static, 1-L glass beakers

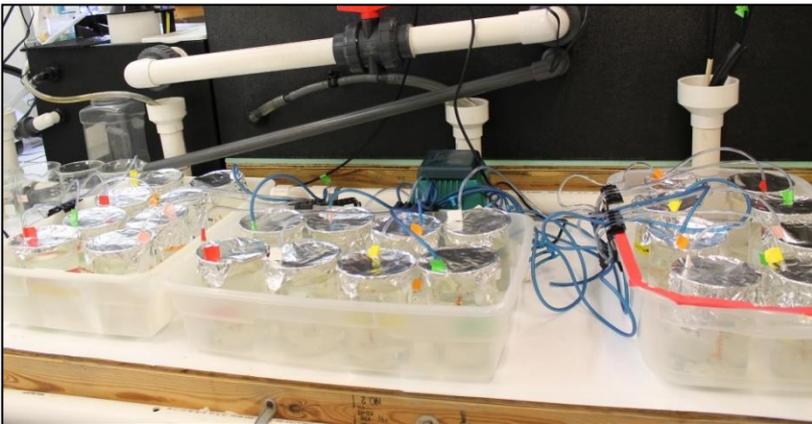
Conditions

Dark, 19°C, 36 ppt salinity
Constant aeration

Water samples collected at $t = 0$

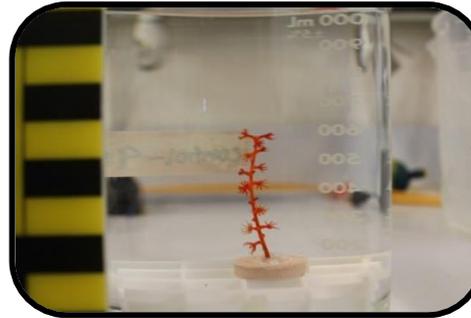
**Hydrocarbons and DOSS
measured**

Health scored daily



Health Scoring

- Photographed and scored at 5 time points:
 - 0, 24, 48, 72, 96 hrs
- Health scores from 0-5 (DeLeo et al., 2016)
 - % of live polyps and remaining tissue
 - Rank 4 or 5 = > 50%
 - Rank 3 = 50%
 - Rank 1 or 2 = < 50%
 - Rank 0 = Dead
 - Loss of color
 - Sloughing of tissue/mucus presence
 - Exposed skeleton



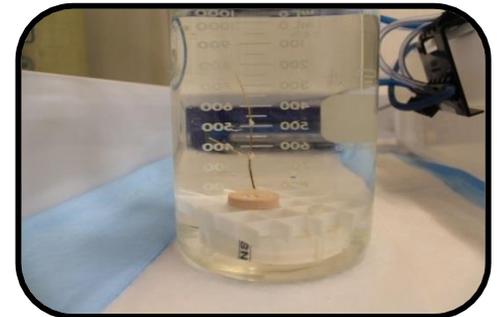
t= 0 h, Health= 5



t= 24 h, Health= 3



t= 48 h, Health= 1



t= 96 h, Health= 0

Chemical Analysis

DeLorenzo et al., *in prep*

Polycyclic Aromatic Hydrocarbons (PAHs) and Total Extractable Hydrocarbons (TEHs)

- Liquid/Liquid extractions w/ dichloromethane and hexane
- Concentration and Silica cleanup
- Analyzed by Gas chromatography mass spectrometry

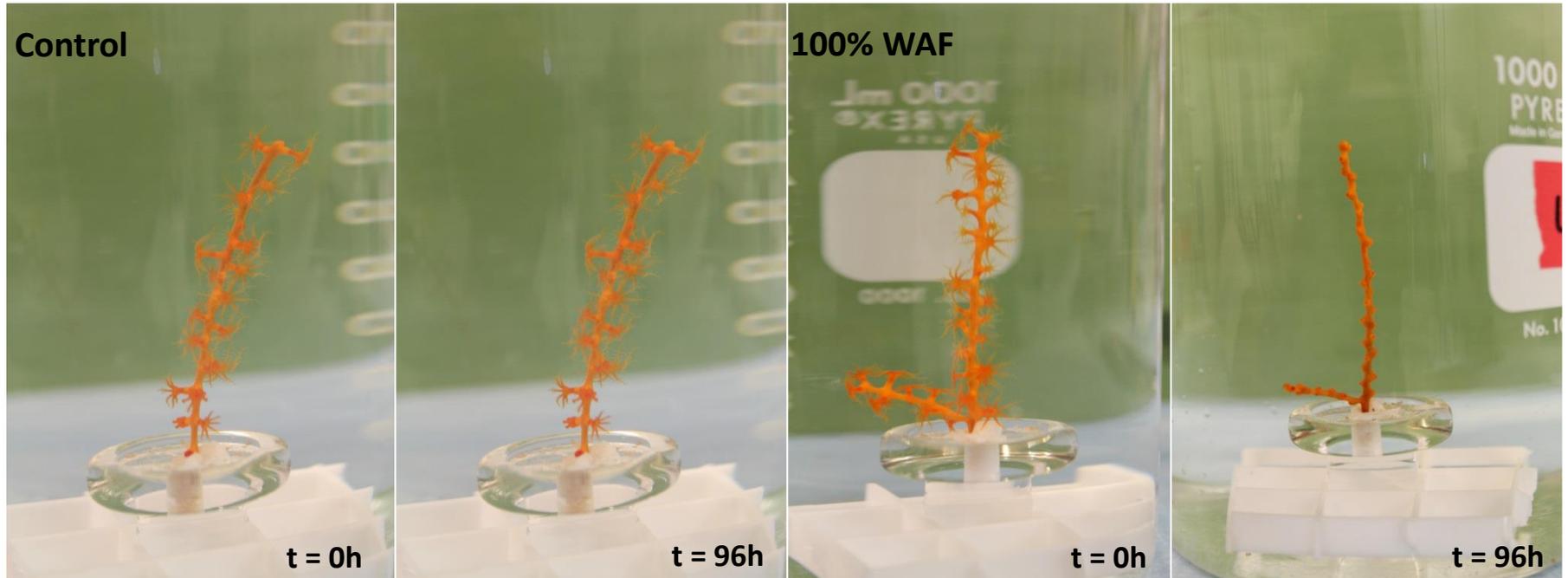
Diethyl Sodium Sulfosuccinate (DOSS)

- Dilution to calibration ranges
- QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) Extraction w/ acetonitrile
- Filtration and Concentration
- Analyzed using liquid chromatography tandem mass spectrometry
- Used to calculate amt. of Corexit (17% DOSS)

Statistical Analysis

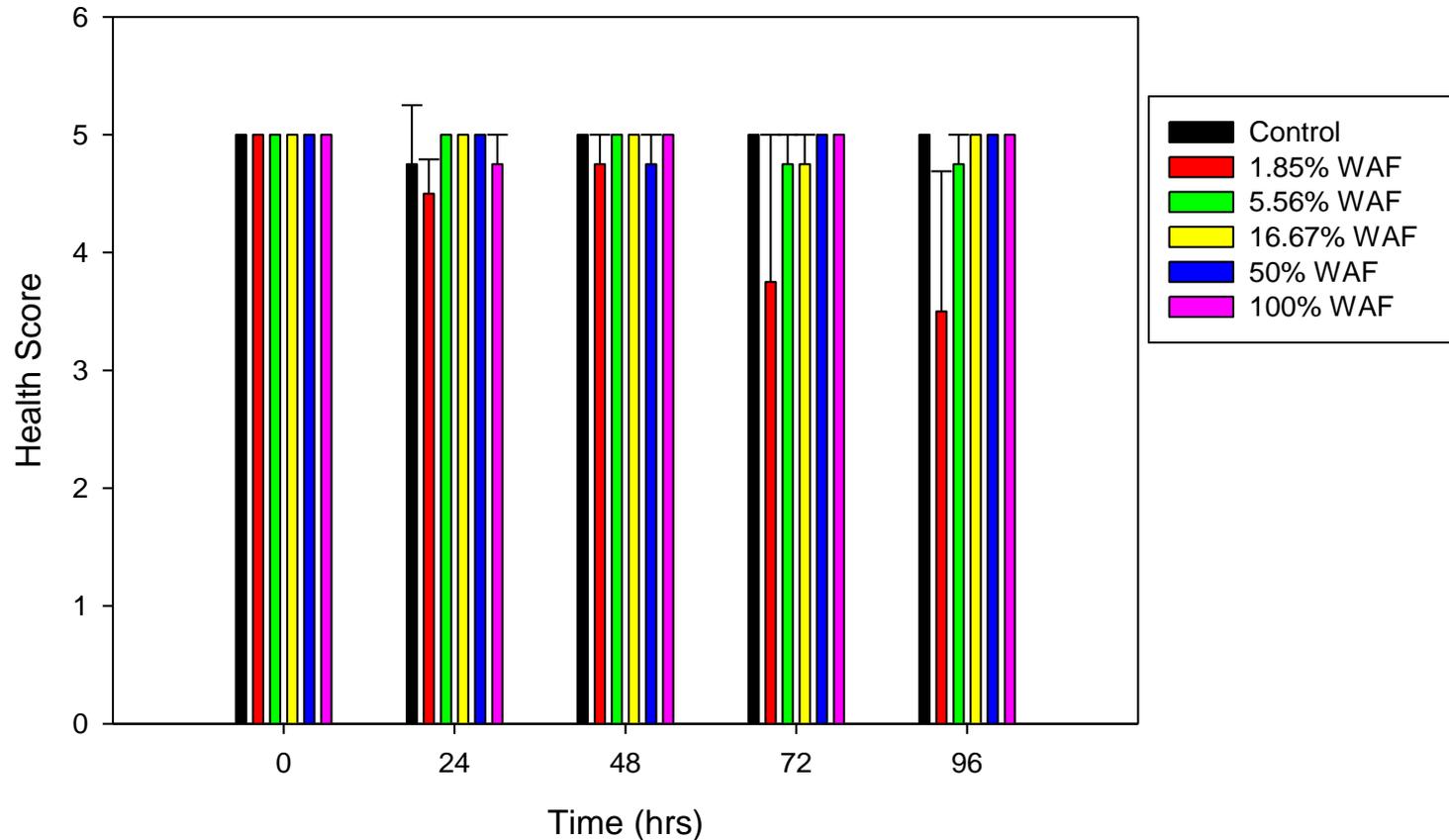
- Health scores averaged at each time point
- Kruskal-Wallis tests (*JMP*)
 - Wilcoxon Pairwise Comparisons (*JMP*)
- Median lethal toxicity (LC50) at 96 hours
 - TEHs and Corexit concentrations
 - PROC PROBIT analysis (*SAS*)

WAF-treated fragments



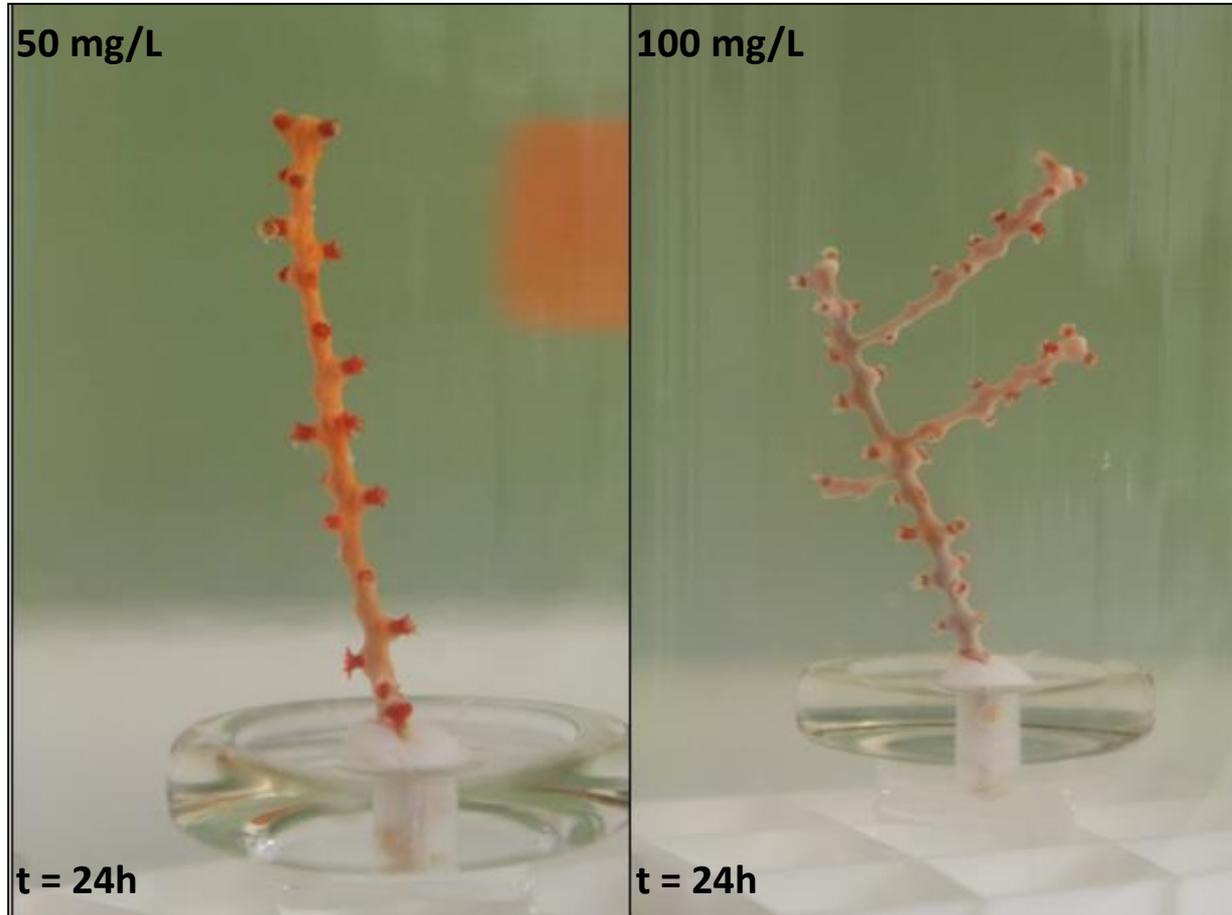
No significant health decline in any treatment group after 96 hours, other than natural polyp retraction

No health decline in WAF treatments



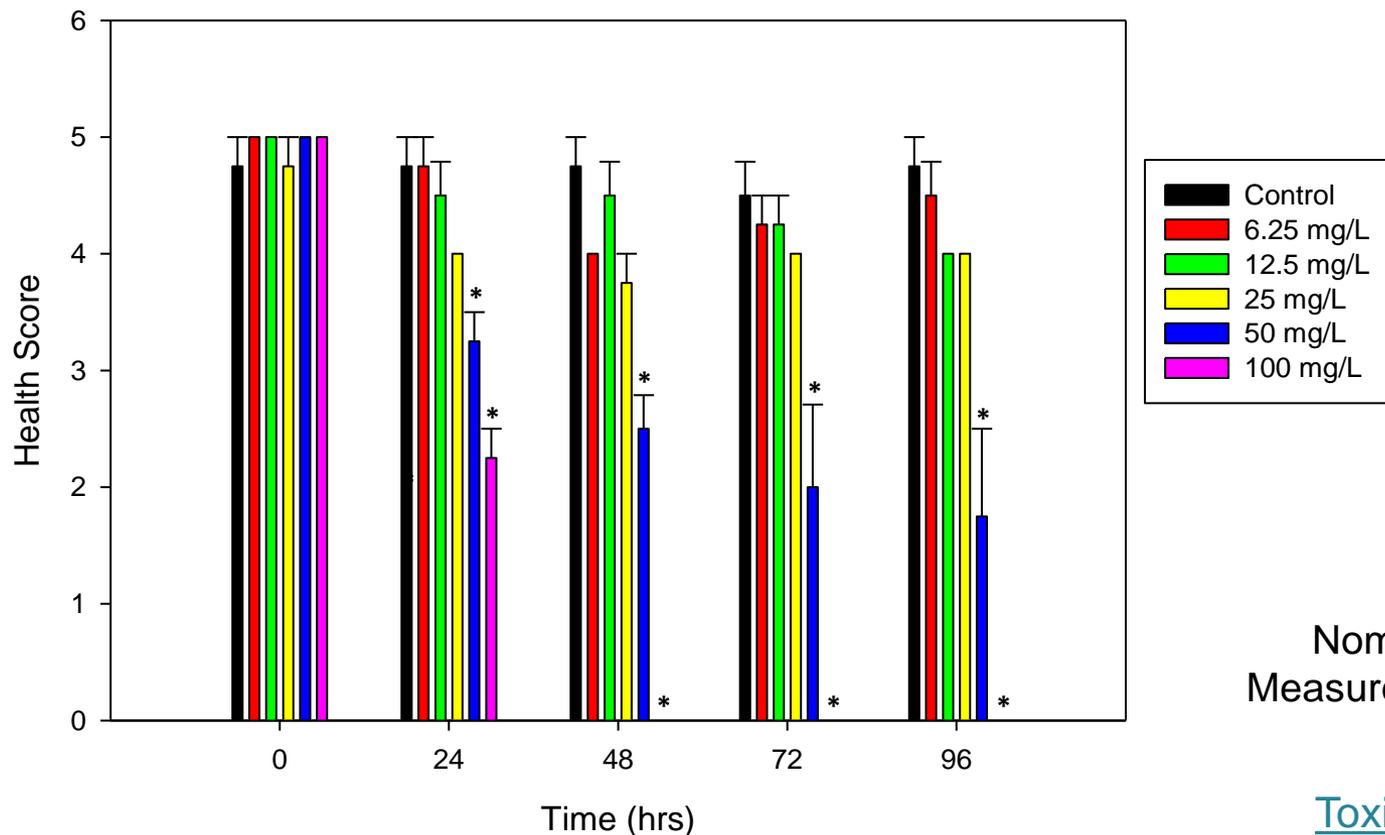
No significant decline in health at any time point

Corexit-treated fragments



Severe health declines after 24 hours at two high-concentration groups
Complete mortality after 48 hours at highest concentration group

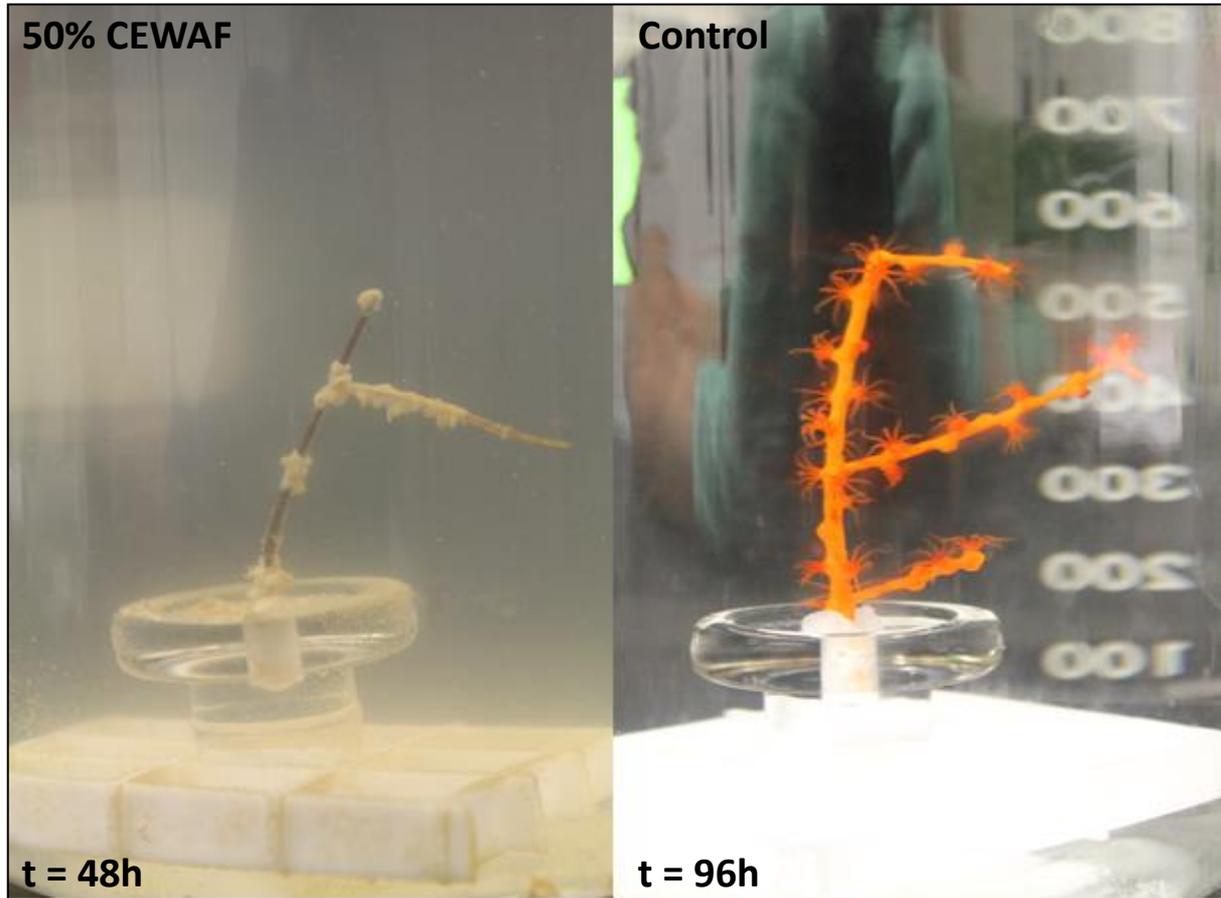
Significant effect of Corexit exposure



LC50s
Nominal: 51.17 mg/L
Measured (Cxt): 70.27 mg/L

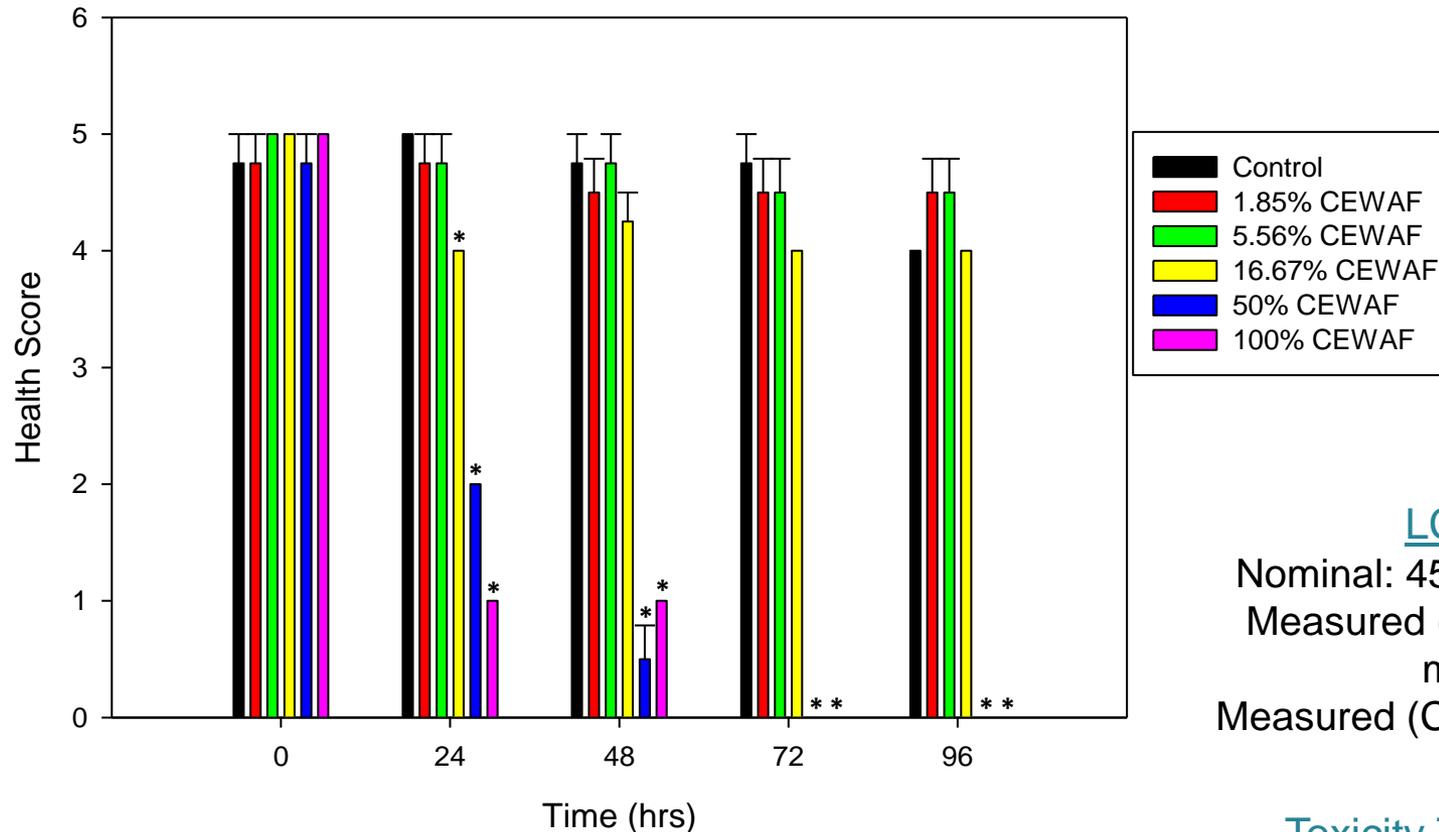
Toxicity Thresholds
67.1 mg/L Corexit

CEWAF-treated fragments



Mortality observed within 48 hours
Complete mortality after 72 hours at both the high-concentration groups

Significant effect of CEWAF



LC50s

Nominal: 45.58% CEWAF

Measured (TEHs): 45.86
mg/L

Measured (Cxt): 41.04 mg/L

Toxicity Thresholds

42.84 mg/L Corexit

49.64 mg/L TEH

Summary

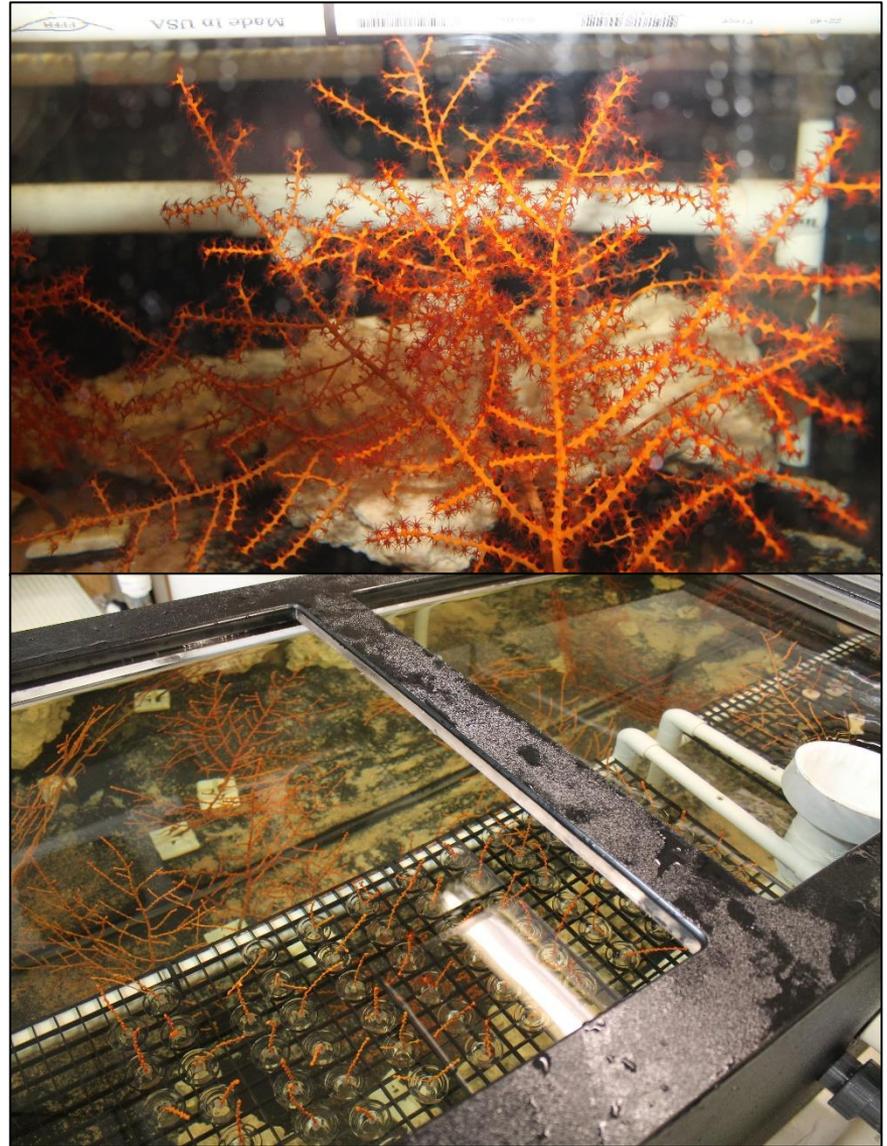
- Molecular and morphological evidence suggests *Swiftia* sea fans in nGoMx are in fact *S. exserta*
- Haplotypes do not appear to be homogenously distributed throughout the 3 regions examined
- *Swiftia exserta* is more vulnerable to treatments that include Corexit than to oil-only WAF treatment



Significance

- First toxicity threshold established for a mesophotic coral species
- Results are consistent with studies that show treatments with dispersant are more detrimental than oil-only treatments (e.g. DeLeo et al., 2015; Goodbody-Gringley et al., 2013; Rico-Martinez et al., 2012 Epstein et al., 2000)
- Provides evidence of vulnerability to chemical contaminants and should therefore inform scientists & managers in the event of a future oil spill
- *Swiftia exserta* DNA suggests regional variability, which is important to understand for conservation efforts

Great experimental animals!



Acknowledgements

Thesis Committee

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