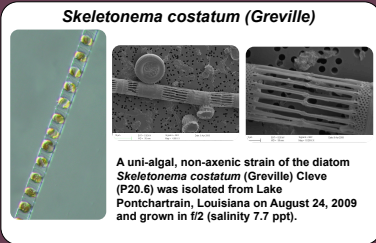


The Effects of Light Slop Crude Oil on the Growth of the Diatom *Skeletonema costatum* (Greville) Cleve



Ngan K. Nguyen¹, Maria Calzada² and James L. Wee¹

¹Dept. of Biological Sciences, ²Dept. of Mathematical Science Loyola University New Orleans, New Orleans, LA 70118



A uni-algal, non-axenic strain of the diatom *Skeletonema costatum* (Greville) Cleve (P20.6) was isolated from Lake Pontchartrain, Louisiana on August 24, 2009 and grown in f/2 (salinity 7.7 ppt).

Abstract

With the increase in maritime transport of chemicals, marine environments have become increasingly threatened by toxins. Crude oil spills in particular can cause widespread ecosystem deterioration as demonstrated by the Deepwater Horizon Oil Spill. This study focuses on the effects of Light Slop crude oil on the growth of the diatom, *Skeletonema costatum* (Greville) Cleve, using a strain isolated from Lake Pontchartrain. Aged seawater from Lake Pontchartrain (salinity = 7.7 ppt) was enriched with nutrients and filtered to make f/2 culture media. Oil contamination was introduced to the culture media via emulsification (1:9, 24 hours, 200 rpm). Experimental treatments included uncontaminated f/2 culture media, oil-emulsified f/2 and oil-emulsified f/2 diluted with uncontaminated f/2 to produce culture media containing 25%, 50% or 75% of oil-emulsified f/2. Preliminary studies indicate that optical density at $\lambda = 730$ nm correlate to cell counts, and hydrocarbon chain lengths of undiluted, oil-emulsified f/2 decrease during the course of the experiment. To observe growth of cultures, OD₇₃₀ readings were recorded on alternate days for ten days. The slopes of the growth curves were compared among the different experimental treatments from day 4 to day 10. Uncontaminated f/2, 25% and 50% treatments showed similar growth curves; the 75% treatment exhibited inhibited growth before recovering at day 4, while no growth occurred in the undiluted, oil-emulsified f/2 treatment. The slopes for the uncontaminated f/2 culture media and 25% treatment were the same ($p < 0.001$), suggesting that oil contamination in the 50% treatment can inhibit growth.

Methods

The water-soluble fraction of Light Slop crude oil was introduced to the culture media via emulsification (1:9 oil-to-culture media, 200 rpm, 24 hours, 24 °C). The water-insoluble crude oil fraction (i.e. organic layer) was removed and discarded. The aqueous layer (designated oil-emulsified f/2) was diluted with uncontaminated f/2 culture media to yield the percent oil-emulsified f/2 in each experimental treatment. Optical density at $\lambda = 730$ nm was measured on alternate days.

Acknowledgements

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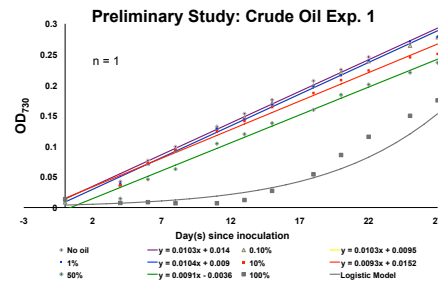


Figure 1. Uncontaminated f/2 is designated as "No oil." Experimental treatments, 0.1%, 1%, 10% and 50% denote the dilution level of oil-emulsified f/2 with uncontaminated f/2. Growth curve for undiluted oil-emulsified f/2 is designated as 100%. Analysis of covariance of days 4 - 13 indicate that the slopes for treatments 0%, 0.1%, 1% and 10% were not different but different from the 50% treatment (p -value = 8.28×10^{-9}).

Preliminary Study: Crude Oil Exp. 2

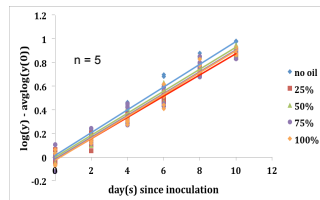


Figure 2. The transformed growth curves of experimental treatments in Oil Exp. 2. Analysis of covariance shows that the 50%, 75% and 100% treatments were not different at the 5% significance level but different from the 0% and 25% treatments.

➤ **Conclusion:** The sterile filtration of the oil-emulsified f/2 seemed to have removed crude oil substance(s) that inhibited cell growth, creating the inconsistencies between Crude Oil Exp. 1 and 2.

Hydrocarbon chains/ Metals/Anion	0% Treatment		100% Treatment	
	Day 0	Day 10	Day 0	Day 10
C12-C28 (4.7 mg/L)	<4.7	<4.7	<4.7	<4.7
C28-C35 (4.7 mg/L)	<4.7	<4.7	<4.7	<4.7
C6-C12 (4.7 mg/L)	<4.7	<4.7	5.8	<4.7
C6-C35 (4.7 mg/L)	<4.7	<4.7	5.8	<4.7
Arsenic (4.00 µg/L)	<4.00	<4.00	<4.00	<4.00
Nickel (4.00 µg/L)	<4.00	<4.00	<4.00	<4.00
Vanadium (4.00 µg/L)	<4.00	<4.00	<4.00	4.29
Sulfate (100 mg/L)	514	500	515	499

Table 1. Sub-samples of the 0% and 100% treatments were obtained during Crude Oil Exp. 2 for water chemistry analysis. The yellow, highlighted values indicate notable data points. The Day 0, 100% treatment started out with more C6-C12 hydrocarbon chains but decreased to undetectable levels at the end of the experiment. Both treatments started with a high level of sulfate and ended with a slightly lower sulfate level. The vanadium level for the 100% treatment went from undetectable to 4.29 µg/L (detectable limit at 4.00 µg/L), a trend absent in the 0% treatment.

Preliminary Study: Unfiltered vs. Filtered oil-emulsified f/2

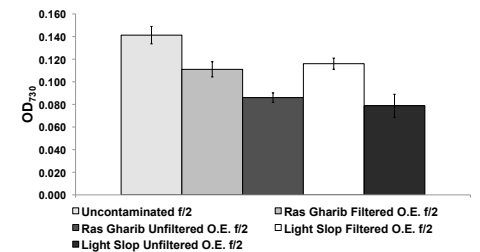


Figure 3. Two crude oils were used to test whether sterile filtration of oil-emulsified f/2 affected the results of Crude Oil Exp. 2. OD₇₃₀ of the experimental treatments were measured on the final day of the study. More growth was observed in the filtered Light Slop and Ras Gharib oil-emulsified f/2 than the unfiltered Light Slop and Ras Gharib oil-emulsified f/2.

Crude Oil Exp. 3

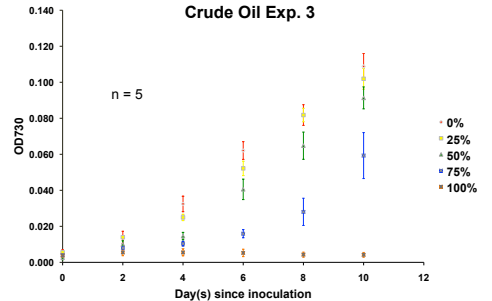


Figure 4. A gradient effect was observed where increases in oil contamination translated to greater growth inhibition. The 100% treatment showed no sign of growth during the study. Analysis of covariance for the slopes of treatments 0%, 25% and 50% from day 4 to day 10 showed that treatments 0% and 25% were the same but different from the 50% treatment ($p < 0.001$).

Summary

➤ Sterile filtration of oil-emulsified f/2 affected the results in the preliminary studies, suggesting that filtration may have removed growth-inhibitory crude oil substance(s).

➤ Oil contamination at levels seen in 0.1%, 1%, 10% and 25% of oil-emulsified f/2 do not affect culture growth of *S. costatum* (Greville) Cleve.

➤ Oil contamination in 50%, 75% and 100% of oil-emulsified f/2 affects the growth of *S. costatum* (Greville) Cleve.

➤ The results of this study suggest the level of crude oil contamination influences algal growth and may make it easier to investigate cellular responses to crude oil contamination.