## Mussel Oiling and Genetic Response to the April 2020 Valdez Marine Terminal Spill: Executive Summary

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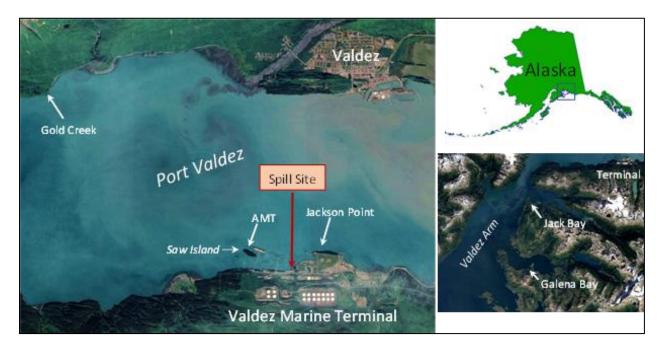
The full final report for this project has been prepared for publication in a peerreviewed journal.

The opinions expressed in this council-commissioned report are not necessarily those of Prince William Sound Regional Citizens' Advisory Council.

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## **EXECUTIVE SUMMARY**

On April 12, 2020, a minor oil spill occurred at the Valdez Marine Terminal (Figure 1) whereby an estimated 1,400 gallons (~34 barrels) of Alaska North Slope (ANS) crude oil overflowed from a sump well and subsequently reached the shoreline, creating slicks and necessitating a full-scale marine response in Port Valdez, Alaska (Figure 2). Recognizing a spill-of-opportunity, the Prince William Sound Regional Citizens' Advisory Council's (PWSRCAC) Scientific Advisory Committee initiated a special project to measure oiling and genetic response of exposed mussels. Mussel samples were taken in a time series over a 7-week period, starting at 19 days post-spill. Most samples were collected at the spill site just outside the terminal's small boat harbor. Other mussels were collected for the Council's annual Long-Term Environmental Monitoring Program (LTEMP) at nearby terminal sites (Saw Island and Jackson Point) out to about 50 days post-spill. At about 50 days post-spill, mussels were also collected from remote unoiled sites in Jack Bay and Galena Bay (Figure 1). Those 2020 LTEMP mussels plus prior LTEMP mussels collected in 2019 serve both as oil spill recovery endpoints and for comparisons to historic background data.

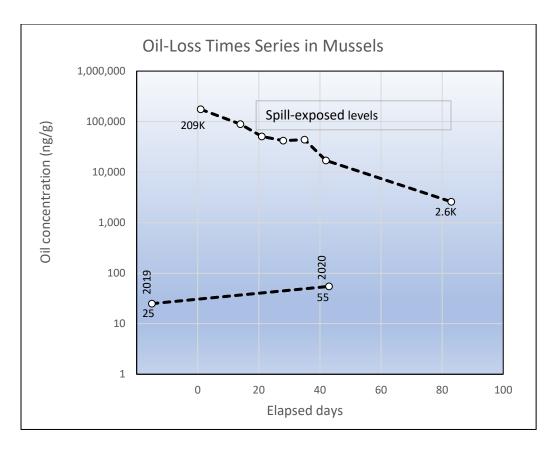


**Figure 1.** Overview of Port Valdez showing the April 12, 2020 intertidal spill location at the Valdez Marine Terminal. Mussels were sampled at the spill site, Jackson Point, Saw Island (AMT), and the control station at Gold Creek 6 km to the northwest. Regional background samples were also collected at Jack Bay and Galena Bay (lower right inset) on June 20, 2020.



**Figure 2.** Containment booms placed around the spill site and in adjacent waters. Saw Island in upper left background adjacent Berth 5 tanker. Image from Alyeska Pipeline Service Company.

Chemical analyses of mussels over time (Figure 3) showed the expected decrease of total hydrocarbons in tissue. Elapsed days in Figures 3 and 5 refers to days from this study's start, but it is important to note, sampling day 1 was 19 days post-spill. By the 40-day mid-point sampling, 2020 LTEMP mussels were approaching 2019 background levels but still held a trace of the spilt oil, while the spill site mussels were 1,000 times more contaminated. The extended deployment of containment booms at the spill site through October 2020 and increasingly weathered chemistry profiles suggest that continued low exposures from sheening continued through at least July, the time of the last mussel collections in this study. By late July when the last samples were collected, the spill site mussels were still 100 times above the 2019 background concentrations.



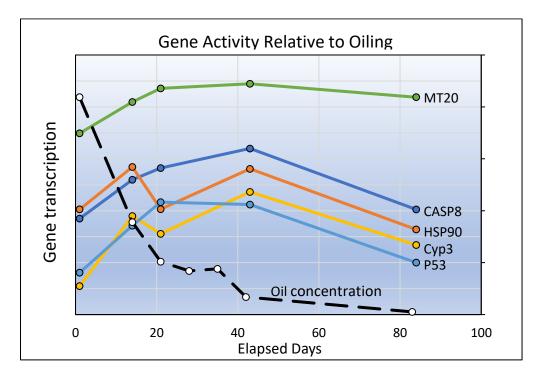
**Figure 3.** Mussel oiling or chemistry shows consistent 100-fold decrease during the sampling period at the spill site but did not reach background levels of 2019 or 2020 LTEMP samples.

When a mussel is exposed to a toxic foreign substance (oil), the animal must somehow deal with it. To survive, the mussel will modify various physiological processes to reduce stresses and mitigate or eliminate the toxin. Exposure to oil is physiologically stressful, with effects including hypoxia (low oxygen), inflammation and immunity issues, and balancing energy needs while detoxifying and eliminating the foreign compounds. Each physiological need requires regulating specific gene activity by boosting or dampening the conversion of a gene's DNA message into protein (transcription). In this project, we measured the transcription of 14 genes, including five directly linked with detoxification processes (Figure 4), and found alterations associated with oil exposure.

Relevant gene activity showed a general trend, with increased transcription lagging behind tissue hydrocarbon concentrations (Figure 5). Transcription levels peaked after the hydrocarbon levels were partially depleted. These results were consistent with other studies where gene transcription was initially inhibited following contaminant exposure. This suggests that the mussels were unable to initially maximize transcription for detoxifying the oil. In addition, by the study's end, with oil still evident in the tissues, gene transcription had not yet completely returned to background levels seen in LTEMP mussels sampled in 2019.

Gene	<b>Biological Process</b>	Environmental Interaction
Metallothionein 20 (MT20)	Detoxification	Contaminants - metals
Caspase 8 (CASP8)	Programmed Cell Death, Necrosis, Inflammation	Pathogens, Contaminants
Heat shock protein 90 (HSP90)	Thermal Stress	Temperature, Pathogens, Contaminants
Cytochrome P450, family 3 (Cyp3)	Detoxification	Contaminants
Tumor protein 53 (P53)	Programmed Cell Death	Contaminants

**Figure 4.** Five genes linked to detoxification processes (related to oil exposure), the primary biological processes they are associated with, and what types of environmental interactions are known to affect their transcription.



**Figure 5.** Transcription levels in 5 genes, directly linked to detoxification (solidcolored lines), in mussels from the spill site. Tissue chemistry (dashed line) diminished throughout the study. Note the lag in gene response, with transcription initially low, then peaking mid-study and subsequently dropping off, whereas oil in tissues consistently decreased. This study has provided a unique opportunity to relate mussel hydrocarbon burdens with gene transcription profiles. In previous years, only the hydrocarbon levels would have been reported and, if elevated, assessed against theoretical toxic-effects levels. However, the addition of gene transcription allows detection of physiological effects in the mussels weeks after hydrocarbon levels have dropped. Our novel findings demonstrate the merits of combining chemistry and genetics to evaluate the extent and persistence of spill effects.

In consideration of the advances made and insights gained, we feel further analyses are warranted. In this project, gene transcription provided a significant advance in our understanding of spill effects. However, this approach needs further development. Specifically, our archived mussel samples can be re-analyzed to obtain the full suite of transcribed genes (transcriptome), quantifying approximately 10,000 genes in contrast to the panel of 14 genes used in this study. Our findings would help to design improved monitoring programs and to better assess spill impacts. We also note that these data are not just applicable to Alaska marine environments. Publishing these methods and interpretations has the potential to globally inform other researchers and regulators regarding contaminant impacts and study designs for discharge or spill assessment programs.

## Recommendations for future monitoring and spill response

- The archived oiled and unoiled mussels should be analyzed for the full transcriptome (i.e., the complete suite of genes transcribed by the organism). Only 14 genes were considered in this study but there are many others that could be analyzed. Comparing exposed versus unexposed mussel response would identify the most appropriate genes for monitoring future oil spills.
- Chemical and genetic methods should be used in future assessments of acute and chronic oil pollution. Monitoring programs which include both body burdens of chemicals and gene transcription of mussels show tremendous benefit as an oil spill, damage assessment approach.
- Additional samples collected from the spill location in 2021 should be analyzed to determine if contamination and transcription levels have returned to normal background levels for Port Valdez.
- A pilot study of seasonal transcription assays would be useful to understand normal baseline expression for monitoring programs, prior to spill events.