Executive Summary: Effects of the April 2020 oil spill detected in study of mussel genes

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Introduction

On April 12, 2020, a minor oil spill was reported at the Valdez Marine Terminal (VMT) in Port Valdez, Alaska. An estimated 1,400 gallons (~34 barrels) of Alaska North Slope (ANS) crude oil overflowed from an onshore sump well and subsequently reached the shoreline, creating slicks and necessitating a full-scale marine cleanup response. Recognizing a "spill of opportunity," the Prince William Sound Regional Citizens' Advisory Council's (PWSRCAC) Scientific Advisory Committee (SAC) initiated a special project to measure oil exposures and genetic response in shoreline mussels from this spill.

This executive summary report builds on previous work conducted by the PWSRCAC to monitor the environmental impacts of the April 2020 oil spill. That work was reported in two prior reports: (1) "Mussel Oiling and Genetic Response to the April 2020 Valdez Marine Terminal Spill: Executive Summary" dated August 2021, and (2) "Mussel Chemistry and Transcriptomic Response after a Minor Alaskan Oil Spill" dated September 2021. The biggest difference between the previous work and the new results presented here is that only 14 mussel genes were analyzed initially, whereas this follow-up study evaluated more than 7,000 blue mussel genes for oil exposure effects.

The goals of the project were to determine:

1) How soon do mussels purge themselves of oil and return to background levels?
2) What genes are turned on or off in response to oiling and can they be used diagnostically for detecting or tracking future spills?
3) Does the gene activity of mussels indicate they are impacted by oil spills longer than indicated by standard hydrocarbon chemistry analysis?
4) Can the genetic response of mussels be used to tell the difference between exposure to ANS crude versus harbor oil-derived contaminants?

In summary, the initial work considering 14 genes and the additional analysis looking at more than 7,000 blue mussel genes demonstrate the merits of combining hydrocarbon chemistry and genetics to evaluate the extent and persistence of oil spill effects. Using gene transcription and hydrocarbon analyses together enabled detection of physiological effects persisting in the mussels as hydrocarbon levels

dropped. Our novel findings demonstrate the benefit of combining chemistry and genetics to evaluate the extent and duration of spill effects. Recommendations are provided at the end of this report that could be used by PWSRCAC to incorporate genetic methods as a regular part of the Council's Long-Term Environmental Monitoring Program (LTEMP).

Methods & Results

Starting 18 days after the initial spill (4/30/2020), mussels were sampled for 15 weeks until mid-August. Most mussels were collected at the spill location just outside the VMT harbor (Figure 1). Mussels were also collected at sites sampled annually for the Council's LTEMP including Saw Island and Jackson Point (Figure 2). About 40 days post-spill, mussels were collected from remote unoiled sites in Jack Bay and Galena Bay to serve as clean references, as well as from the entrance of the Valdez Harbor Figure 2). The Valdez Harbor mussels were collected to understand how the genetic response of mussels exposed to oil pollution from a harbor may differ from mussels exposed to ANS crude oil.

Figure 1. Containment booms placed around the spill site (red arrow) and in adjacent waters at the Valdez Marine Terminal. Saw Island is in the background upper left, adjacent to a Berth 5 tanker. Image from Alyeska Pipeline Service Company.

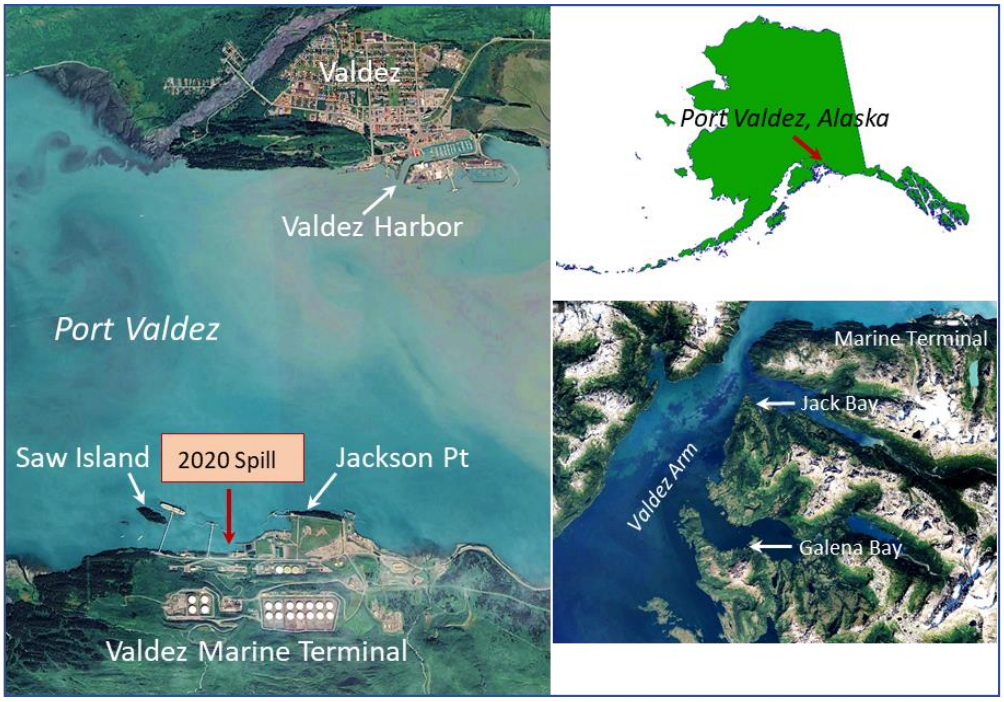

Figure 2. Overview of Port Valdez showing the April 12, 2020 intertidal spill location at the Valdez Marine Terminal. Mussels were sampled at the spill site and LTEMP sites at Jackson Point and Saw Island. Regional "background" mussel samples were collected at Jack Bay and Galena Bay, and at the entrance to the Valdez Harbor.

Mussel oil exposure from this spill was measured using traditional hydrocarbon-chemistry methods standard for LTEMP and reported here as individual and total polycyclic aromatic hydrocarbons (TPAH). As expected, initial TPAH concentrations were extremely high after the spill and then declined towards lower levels over the course of the 111-day project (Figure 3). Day 1 of this study (shown as "elapsed days" in Figure 3) was 18 days after the spill occurred. Chemistry analyses were conducted on mussel samples until day 82 of the study. On day 41, the spill-site mussels were still 300 times more contaminated than standard LTEMP mussels. At the project's final sampling in July 2020 (day 82), concentrations in spill-site mussels remained 100 times greater than the 2019 background LTEMP concentrations, suggesting that intermittent, low-sheening exposures may have continued through, and likely after the end of this effort to specifically monitor the impacts of the April 12th spill. That hypothesis is supported by visual observations from Alyeska personnel who observed small amounts of sheening from the spill site during higher than average tides, well after the spill occurred.

One of three spill-site mussel samples collected during routine LTEMP sampling a year later (June 2021) showed near-background level hydrocarbons (101 nanograms per gram or ng/g in Figure 3) with evidence of oil but not necessarily from the spill. In other words, by July 2021, concentrations of oil in the spill site mussels had returned to low level, baseline concentrations.

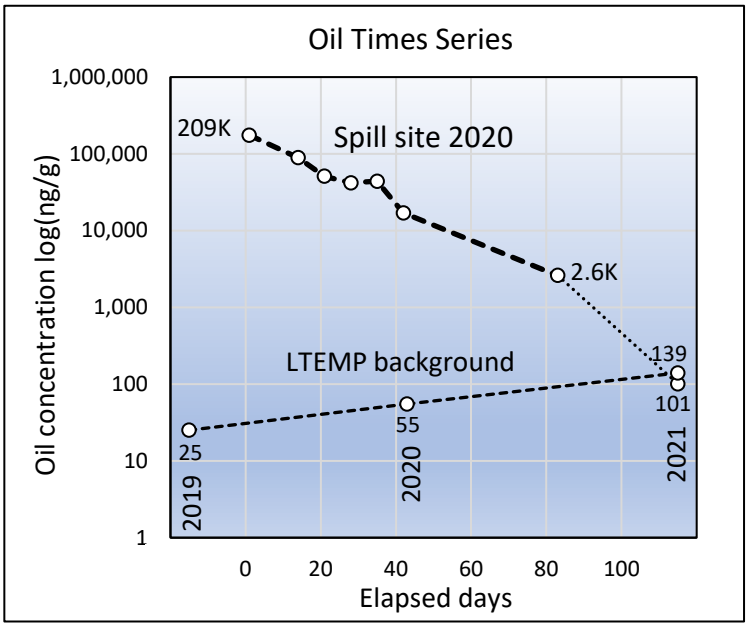

Figure 3. Concentrations of oil in mussels declined 80-fold during the 82-day sampling period from April 30 to August 19, 2020. However, the concentrations in August 2020 (day 82) were still above levels measured during regular LTEMP sampling in 2019 and 2020. By July 2021, oil concentrations in the spill site mussels returned to low, near-normal LTEMP levels (139 ng/g measured at spill site vs. 101 ng/g measured at regular LTEMP sites near the Valdez Marine Terminal).

Using genetic techniques to monitor environmental impacts on organisms is becoming more commonplace, and through this project, SAC is trying to understand if such techniques should become a part of the regular LTEMP scope of work, which has historically relied on hydrocarbon chemistry methods to monitor oil contamination. In this project, transcriptomic techniques were used to assess how the oiled mussels were responding to the spill. Transcriptomics involves the study of RNA molecules being produced within a cell. Transcription is the process in which DNA in the genes is converted into RNA molecules, which are then used to create specific proteins within a cell. By measuring which RNA molecules, and how many of

them, are being produced inside an organism, transcriptomics can be used to understand how that organism is genetically responding to environmental stressors such as oil spills.

Mussel gene transcription activity was assessed twice in relationship to the April 2020 oil spill. Initially only 14 genes were analyzed (2020), and then the scope was expanded to consider all the mussel genes (this study). During the 2020 initial study of the oiled mussels, the tissue samples were analyzed using an abbreviated suite of 14 genes that were previously used for similar projects in the region, funded in part by the *Exxon Valdez* Oil Spill Trustee Council. In the initial study, five genes responded to oiling with similar time series patterns (Figure 4). There was an initial lag followed by increased expression that eventually dropped back to near-reference levels by mid-August. In contrast, oil concentrations fell from very high to significantly lower at that time, but still 100x background amounts (Figure 3). Although oil concentrations in the mussels had declined significantly by August 2020, genetic response to the oil spill was still evident.

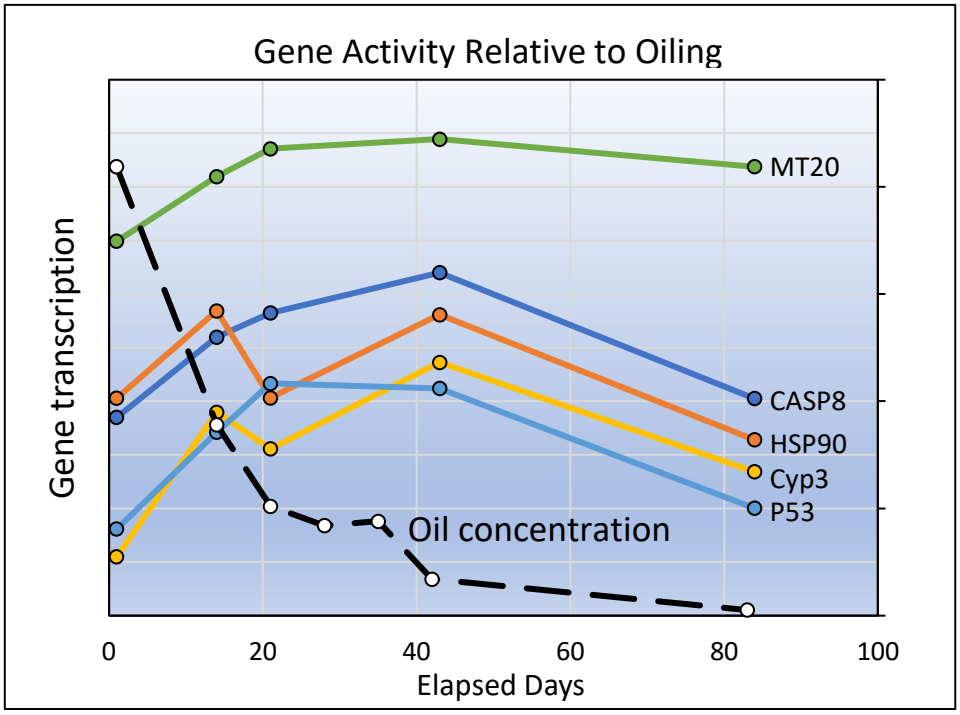

Figure 4. Initial study results showing gene activity trends as oiling levels decrease. Solid colored lines are five genes from original 14 gene panel (Bowen et al., unpublished data).

Following the 2020 initial project, the SAC approved using these same oiled mussel samples for an expanded look at the complete transcriptome (i.e., all the genes being expressed by the mussels at the time of the spill). The goal was to assess all genes and then focus on those genes behaving differently among the spill site, the Valdez Harbor, and the "clean" reference site mussels (Jack and Galena Bay, Figure 2). The key question this expanded transcriptomics study was attempting to answer was "could the genetic response of mussels be used to tell the difference between exposure to ANS crude vs versus harbor oil-derived contaminants?"

Analyzing and interpreting the complete transcriptome data has been a complicated and challenging task. One issue of working with Pacific blue mussels (*Mytilus trossulus*) is that their genes are less well studied compared to the more globally occurring Mediterranean mussel (*M. galloprovincialis*). Due to the relative lack of data on Pacific blue mussels, Mediterranean mussels were used for the reference DNA dataset. As a result, while the transcription analysis reported over 7,000 *Mytilus trossulus* genes active during the 2020 oiled mussel time series, only 66% of those genes could be identified and attributed to a presumed biological function.

To achieve any success with this superabundance of genetic data, the analytic focus had to be limited to those functions, either biological, cellular, or molecular, that from other studies were attributed to systems known to be impacted by oiling. Further, in the data presented here, the genes of interest were limited to those that displayed expression trends that appeared significantly responsive to oiling. Four approaches were used to examine the mussel transcription data: Venn diagrams, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functionality, Gene Ontology functions, and selected gene trends.

The Venn diagram (Figure 5) shows the overlaps of how many genes had levels of expression in common between sites. "HOTA" represents spill site mussels, "HARA" represents Valdez Harbor mussels, and "BAY1A" represents Galena and Jack Bay mussels. Note that there are genes unique to each treatment group. Some portion of the 360 + 481 genes identified in mussels collected at the spill site (HOTA) whose expression is unique from the reference mussels (BAY1A) are presumed to be related to oiling response (e.g., from ANS crude oil or harbor oiling sources such as spilled diesel fuel).

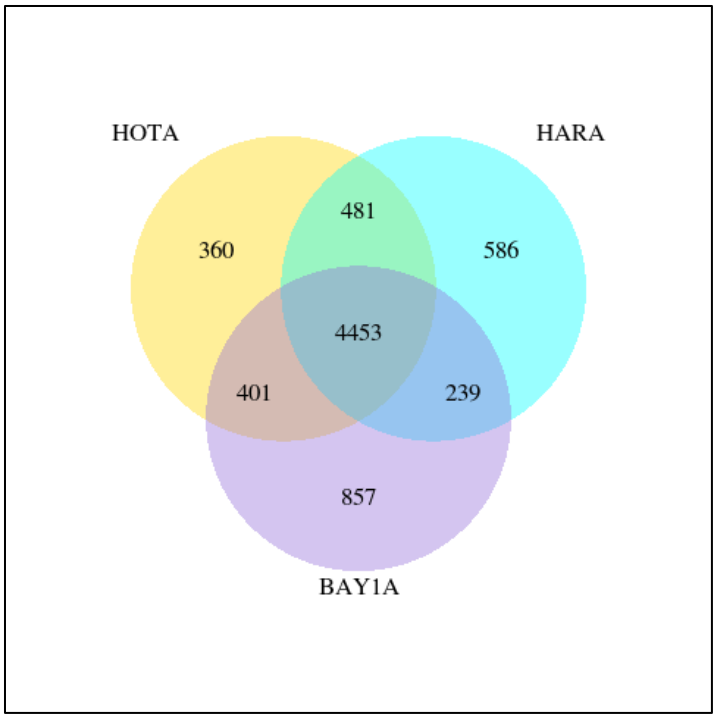

Figure 5. Counts of distinct and common mussel gene expression between sites, from full transcriptome analysis. HOTA is the spill site (see Figure 2); HARA is the Valdez Boat Harbor; and BAY1A is the unoiled Galena and Jack Bay sites.

From another perspective, the field of bioinformatics (i.e., using computers and software tools to understand large and complex biological datasets) has progressed such that most genes can be identified and assigned a presumed function. Two separate bioinformatic approaches, KEGG and Gene Ontology functions, were used to examine the active pathways and functional groupings for oiling effects. These analyses compared each sampling to the unoiled reference sample (BAY1A) and plotted statistical differences or pathway linkages (although both HOTA and HARA were compared to BAY1A, in this report our focus is on results for HOTA). The overall results showed a very dynamic system of gene expression changing throughout the time series.

Finally, from published studies, genes selected by their functional relationship to oil detoxification were plotted and examined for distinctive time trends. From this effort, a "short" list of 50+ prospective genes of potential interest was assembled to address the project objectives. These genes of interest appear within the gene families of Table 1 below. These prospects will require further validation either in another spill-

of-opportunity or better, in a controlled environment (at a toxicity lab or an oil-spill wave tank).

Table 1. Genes identified for potential use in a new transcription. Listed genes were chosen (A) to distinguish between spill-site responses and unoiled reference sites (Jack and Galena Bays) and (B) to potentially differentiate between Valdez Marine Terminal spill site and Valdez Harbor (ANS crude oil versus vessel exhaust and diesel contamination at harbor).

(A) Spill site vs Reference sites

Gene or gene family	Number of genes
ABC (ATP-binding cassette)	8
Glutathiones	18
Heat shock proteins	9
Helicase	26
Immune related	21
Kinesin	11
Meiosis	7
Neurotransmitter	9
Oxidative stress response	1
Cytochrome P450	7
RNA recognition motif	24
General stress	3
Superoxide dismutase	2
Tumor necrosis family	16
Ubiquitin	39

(B) Spill site vs Valdez Harbor

Gene or gene family	Number of genes
ABC (ATP-binding cassette)	13
Glutathiones	16
Heat shock, HSP, chaperone	21
Helicase	1
Immune related	35
Neurotransmitter	11
Cytochrome P450	7
RNA recognition motif	4
General stress	9
Superoxide dismutase	3
Tumor necrosis factor	11
Ubiquitin	49

Conclusions

Several conclusions were reached:

- Lower levels of oil were still present in spill-site mussels by the final sampling in mid-July, 82 days after sampling began. These levels were still 50-100x greater than LTEMP background levels from 2019-2020, which suggests that intermittent sheening possibly was still occurring by the end of sampling.
- From the time series of transcriptome differences:
    - gene activities related to oil detoxification and recovery processes were identified.
    - in multiple pathways, gene activities did not return to reference-site levels, suggesting that recovery from hydrocarbons was not complete by final sampling. This may have been due to the presumed residual sheening.
- Transcriptome differences among the three sites:
    - gene responses known to occur with oil exposure were identified in the spill site vs the unoiled sites.
    - genes that could potentially distinguish between ANS crude oil and harbor contaminants (pyrogenics and diesel) were identified.

In this project, gene transcription analyses have advanced our understanding of spill effects on Pacific blue mussels (*Mytilus trossulus)*. They have provided a unique opportunity to compare transcriptomic responses of mussels from unoiled sites, a spill site (ANS crude oil), and a harbor (various oil-derived contaminants including those from diesel fuel and vessel exhaust). In previous years, only the hydrocarbon chemistry levels would have been reported and, if elevated, assessed against various reported toxic-effects levels.

In consideration of the advances made and insights gained, we recommend implementing transcription analyses as part of LTEMP monitoring and response protocols. However, the approach needs further development. In order to confidently incorporate any of the relevant genes identified in this study into new gene-transcript assay panels, they will need validation, preferably in a controlled or known exposure experiment.

Our findings will help to design improved monitoring programs and to better assess spill impacts and recovery. We also note that these data are not just applicable to

Alaskan marine environments. These methods and interpretations have 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

Research

- If the gene prospects identified in this study are to be incorporated into new gene transcript assay panels, they should be validated in a controlled environment (at a toxicity lab or an oil-spill wave tank).
- Develop a gene assay panel inclusive for two potential oiling events, acute spill vs. routine monitoring.

Continuing LTEMP studies

- The original 14-gene assay panel used in the 2020 initial study responded to this ANS crude spill. While developing a new panel from the prospective list of 50+ genes, continue using the current 14-gene panel for LTEMP monitoring.
- A limitation to this observational study was that the unoiled reference sites were only sampled once during the time series (6/9/2020). Any future study should include controls taken at the same timepoints as the oiled samples in the series.