Briefing for PWSRCAC Board of Directors – January 2022

ACTION ITEM

Sponsor: Austin Love and the Scientific Advisory Committee

Project number and name or topic: 9510 – Long-Term Environmental Monitoring Program

1. **Description of agenda item:** This agenda item is seeking Board approval to provide the United States Geological Survey with a research contribution of $75,555 to genetically analyze blue mussel samples obtained to monitor the environmental impacts of the April 12, 2020 oil spill at the Valdez Marine Terminal.

2. **Why is this item important to PWSRCAC:** The Oil Pollution Act of 1990 instructs the PWSRCAC to "devise and manage a comprehensive program of monitoring the environmental impacts of the operations of terminal facilities and of crude oil tankers while operating in Prince William Sound." The work done under the Council's Long-Term Environmental Monitoring Program has been designed by the Scientific Advisory Committee to fulfill that responsibility mandated by the Act.

3. **Previous actions taken by the Board on this item:**

Meeting	Date	Action
Board	1/23/2020	The Board accepted of the "Port Valdez Mussel Transcriptomics" report by Lizabeth Bowen of the U.S. Geological Survey, dated November 20, 2019, as meeting the terms and conditions of contract number 951.20.06, and for distribution to the public.
Board	5/21/2020	Approval of FY2021 Contracts for Project 9510 LTEMP - The Board approved the following: Authorizing a contract negotiation with Payne Environmental Consultants Inc., for work to be performed under LTEMP, at an amount not to exceed $115,064. Authorizing a contract negotiation with Newfields Environmental Forensics Practice, for work to be performed under LTEMP, at an amount not to exceed $95,807. Authorizing a contract negotiation with the United States Geological Survey, for work to be performed under LTEMP, at an amount not to exceed $65,371. Authorizing a contract negotiation with Oregon State University, for work to be performed under LTEMP, at an amount not to exceed $22,030. Authorizing a contract work to commence prior to the start of FY2021, as approximately $33,000 of these funds will need to be expended in May and June 2020.
Board	5/6/2021	The Board accepted the report titled "Long-Term Environmental Monitoring Program: 2020 Sampling Results and Interpretations," by Dr. James R. Payne and William B. Driskell, dated March 2021, as meeting the terms and conditions of contract number 951.21.04, and for distribution to the public. The Board accepted the report titled "Using Mussel Transcriptomics for Environmental Monitoring in Port Valdez, Alaska: 2019 and 2020 Pilot Study Results", dated February 17, 2021, as meeting the terms and conditions of contract number 951.21.06 and for distribution to the public.
Board	5/21/2021	Approval of FY2022 LTEMP Contracts for Project 9510: The Board Authorized individual contracts with Newfields Environmental Forensics Practice, Oregon State University, and the United States Geological Survey (USGS) with the

aggregate total not to exceed the amount approved in the final FY2022 LTEMP budget ($147,720) for contract expenses, and delegated authority to the Executive Director to enter into individual contracts with the aforementioned consultants; and authorized that the contract work commence prior to the start of FY2022 as approximately $30,000 of these funds will need to be expended in May and June 2021.

4. **Summary of policy, issues, support or opposition:** Since 2019, the Council has been working with Dr. Liz Bowen from the United States Geological Survey to potentially incorporate a new genetic testing method into the Long-Term Environmental Monitoring Program (LTEMP) – that genetic method is technically called transcriptomics. Transcriptomics is a promising new tool to add to LTEMP and in 2019, 2020, and 2021 the Scientific Advisory Committee has advised that the Council conduct transcriptomics monitoring work to serve as a pilot study, the results of which would be used to determine if the Council should continue to use this technique in the long term. Originally, the pilot study was only planned for 2019 and 2020, but then the April 12, 2020 oil spill occurred, providing a unique opportunity to further test the utility of transcriptomics to monitor the environmental impacts of the Valdez Marine Terminal and tankers. The originally planned 2019 and 2020 transcriptomics work has been completed and culminated in a report to the Board titled "Using Mussel Transcriptomics for Environmental Monitoring in Port Valdez, Alaska: 2019 and 2020 Pilot Study Results," which was approved during the May 2021 meeting. While some transcriptomics work related to the April 12, 2020 spill has been completed and the results were informative, this agenda item seeks funding for additional spill-related work, which is designed to try and maximize the utility of transcriptomics for the Council's LTEMP. This funding would be used to try and identify a set of genes in blue mussels that specifically respond to exposures to Alaska North Slope crude oil – as opposed to genes known to respond to generic oil exposure.

Making a research contribution to the United States Geological Survey for this work has significant financial benefit for the Council. By making a research contribution compared to entering into a contract, the Council will avoid paying overhead costs of 51.25% (e.g., this work would cost $114,278, instead of $75,555). Since 2019, Council has made research contributions (totaling $75,131) to the United States Geological Survey to support related transcriptomics work and the results of all those contributions have been successful (i.e., the research and associated report was completed and delivered to the Council). The Finance Committee has expressed concern over research contributions and requested that guidelines regarding future research contributions be developed.

5. **Committee Recommendation:** During their April 29, 2021 meeting, the Scientific Advisory Committee recommended that the Council should implement and prioritize Recommendation # 5 listed in Dr. Bowen's report to the Council titled "Using Mussel Transcriptomics for Environmental Monitoring in Port Valdez, Alaska: 2019 and 2020 Pilot Study Results." That report was approved during the May 2021 Board meeting. Recommendation #5 proposed:

"conducting an experiment with samples from the April 12, 2020, oil spill at the Valdez Marine Terminal (already collected and in our freezer). We would perform a full transcriptome analysis (RNAseq) of mussels exposed to the spill. This would allow for identification of genes specific to the contaminants in a carefully monitored real-world spill event such as the spill from the Valdez Marine Terminal in April 2020. This will increase both the specificity and sensitivity of the gene panel for the needs of the PWSRCAC."

During their October 1, 2021 meeting, the Scientific Advisory Committee reviewed a proposal by Dr. Bowen detailing how recommendation #5 would be implemented, which the committee recommends the Board accept.

6. **Relationship to LRP and Budget:** The Council's 9510 Long-Term Environmental Monitoring Program (LTEMP) is in the approved FY2022 budget and annual work plan. Since the FY2022 LTEMP budget was passed, the scope of work for the transcription part of LTEMP has been expanded as recommended by the Scientific Advisory Committee and subsequently costs for this part of LTEMP became higher than originally budgeted. Therefore, in order to cover the costs of this expanded transcription work, a budget modification is being requested under agenda item 4-8 for LTEMP.

9510—Long-Term Environmental Monitoring (LTEMP)

As of December 10, 2021

FY-2022 Budget	
Original	$154,980.00
Modifications	
Revised Budget	$154,980.00
Actual and Commitments	
Actual Year-to-Date	$37,906.65
Commitments (Professional Services)	$11,553.00
Actual + Commitments	$49,459.65
Amount Remaining	$105,520.35

7. **Action Requested of the Board of Directors:** Provide the United States Geological Survey with a research contribution of $75,555 to genetically analyze blue mussel samples obtained to monitor the environmental impacts of the April 12, 2020 oil spill at the Valdez Marine Terminal.

8. **Alternatives:** The Board could choose to enter into a contract with the United States Geological Survey for this work, resulting in an additional 51.25% overheard charge, for a total contract amount of $114,278. This alternative action would require a budget

modification from the contingency fund to project 651 in the amount of $38,723 to cover the increase.

9. **Attachments:** Research proposal from Dr. Liz Bowen from the United States Geological Survey and collaborators.

Proposal: Transcriptomic analysis of an oil spill response in mussels (*Mytilus trossulus*) in Valdez, Alaska

Lizabeth Bowen[1*], William B. Driskell[2], James R. Payne[3], Brenda Ballachey[4]

1 U.S. Geological Survey, Western Ecological Research Center, Davis, CA 95616

2 Consultant, Seattle, WA

3 Payne Environmental Consultants, Inc., Encinitas, CA

4 U.S. Geological Survey (Emeritus), Alaska Science Center, Anchorage, AK

Prior to a minor spill incident at the Valdez Terminal in April 2020, a pilot study had been conducted in 2019 that looked at transcription in mussels for PWSRCAC relevant sites in Port Valdez and PWS. The study used earlier protocols employed for prior National Park Service (NPS) surveys in south-central AK, which were designed to evaluate various NPS environmental concerns (climate change, acidification, immunity, and inflammation). This same gene panel was used in evaluating mussel response to the Terminal oil spill. Results show that five genes with detoxification functionality responded to the oiling (Figure 1). While only 14 genes were considered for the spill study, there are likely a multitude of others that were responding.

Proposed for this project, the full transcriptome (i.e., the complete suite of genes actively transcribed by the organism) will be analyzed for the archived oiled and unoiled mussels. Differences in their activities would suggest what physiological systems were turned on (or off) in response to oil exposure. It is assumed that, during the spill, the response of mussels with initial TPAH body burdens of >200K ppb would include essential body maintenance functions (homeostasis) as well as detoxification. The time series plot shows a lag in response (gene expression peaking during TPAH decline; Figure 1) that suggests non-observed, homeostatic transcription was likely prioritized before the observed detox genes' activity peaked. A full transcriptome analysis is expected to reveal multi-thousands of active genes.

Why do we care?

The annual LTEMP monitoring program has pursued a traditional chemistry-only approach that analyzes a limited hydrocarbon suite (PAH, SHC and biomarkers). This has been appropriate as most toxicity studies describe effects based only in terms of concentrations of specific oil components or as summed indices such as TPAH43, or more recently, from oil's dissolved or dispersant-enhanced fractions. However, considering the universe of hydrocarbons present in oil (McKenna et al., 2013) that are not accounted for in these classical toxicity studies, the

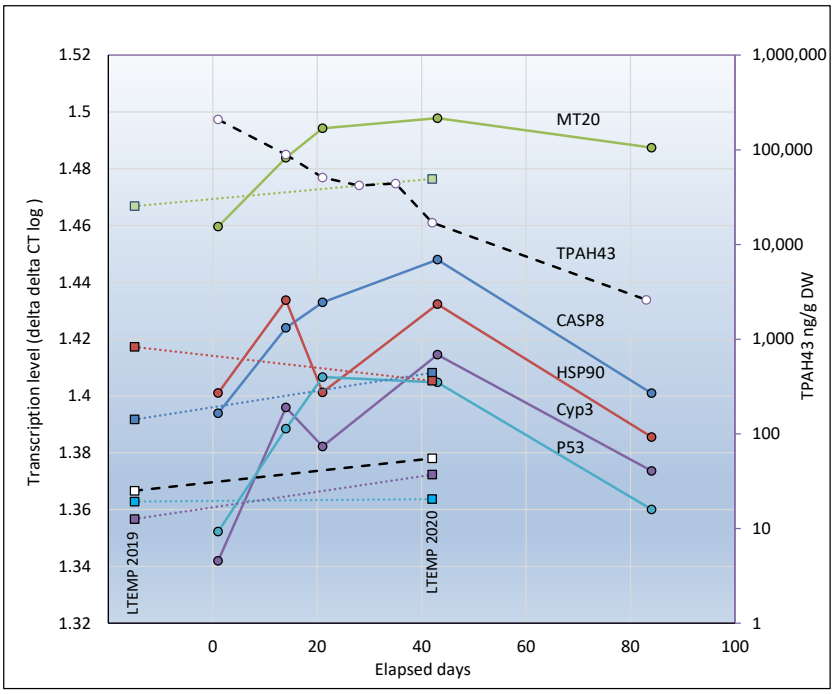

Figure 1. Transcription levels of genes directly linked to detoxification in adductor muscle tissues (solid lines) and TPAH43 tissue concentrations (oil; dashed lines). LTEMP 2019 and 2020 values are from two LTEMP sites depicting average background levels (square symbols connected by dotted lines for genes and a dashed line for TPAH43). From Bowen et al., in submission.

traditional chemistry approach based on summaries of amenable-to-traditional-analyses components as causal proxies, seems myopic. Nor it is yet known explicitly which components are detrimental to an organism. As such, attempting to link effects from just contaminant concentrations can be suggestive but certainly not definitive.

Setting aside the fuzziness in exposure-assessment issues, evaluating effects can be equally enigmatic. It is fundamentally obvious that an organism incurs cellular-level impact from oil exposure but pinning down the exact damage has been an evolving science. After decades of LD50-style testing, there has been great progress in looking at actual physiological impacts (Garmendia 2011, Sørhus, 2020). But in connecting physiological impacts with an organism's phenotypic or population-level impacts, there remains a knowledge gap.

Fortunately, advancements in molecular assessments now allow us to see exactly which homeostatic mechanisms are going awry during exposures. As proposed, linking exposure to functional transcription activity, i.e., the putative physiological function for a gene, can provide tremendous insights into how an organism responds to an environmental stressor. A prime example of this more intimate clarity is the work of Incardona, et al. (2009) demonstrating that heart defects in EVOS oil-exposed herring embryos (Carls et al. 1999) are due to an anomalous genetic expression affecting the sodium ion pump of nerve and muscle cells. Later extensions of this work during the BP Gulf spill (Incardona, et al., 2014 and others) confirmed that this cellular

defect in fish compromises their motility and thus, their robustness to find and capture food or escape predators.

These fish studies exemplify the long-sought goal of connecting the dots between exposure and individual effects, albeit not necessarily evaluating population impacts. This point is conceptually illustrated as a continuum of biological focus (Figure 2). There is no single approach that is comprehensive for all levels of concern or ecological relevance. Transcriptome studies, however, do cross biological levels, reporting subcellular activities representing an organ's response (here, gill and adductor muscle) that suggest an individual organism's status (degree of impact/recovery relative to non-exposed individuals).

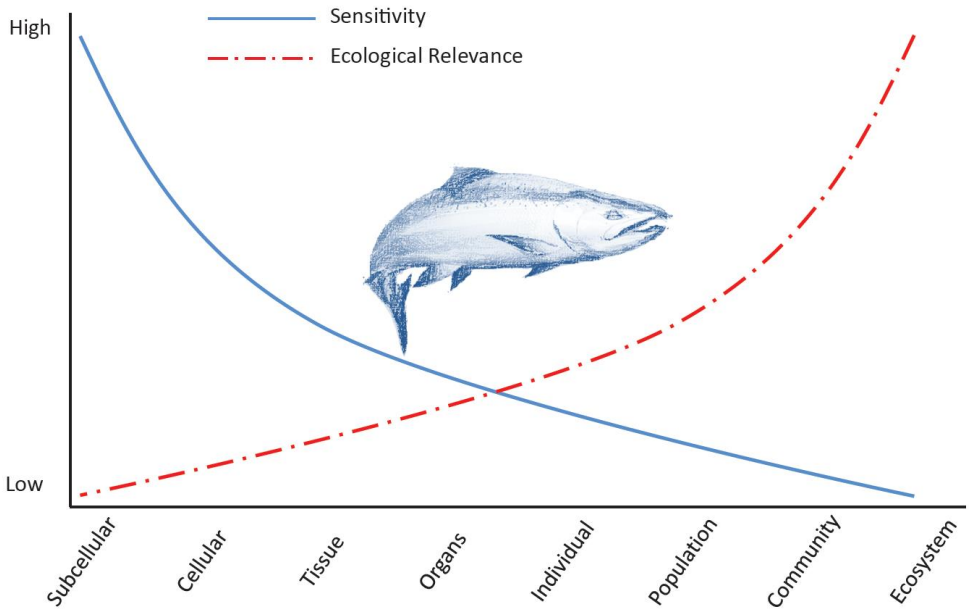

Figure 2. The sensitivity and ecological relevance of toxicological assessments vary depending on the level of biological organization at which tests are conducted. Evaluation of links across multiple levels of biological organization provide for a comprehensive effect-based assessment of perturbations within an ecosystem. From Connon et al. 2019.

For this proposal, knowing the functional suites of genes that get turned on (or off) in oiled mussels will serve several purposes.

- Understanding the full diversity of response. It is assumed that with a limited energy/resource budget, an organism must optimize expenditures dealing with the essential homeostatic needs e.g., respiration, thermal and pH regulation, flushing, feeding, etc., while turning on the AHR response to detoxify xenobiotics. The reported detox transcription lag (Figure 1) supports this paradigm.

- Timing of response. The functional responses appear to change over time as essential issues are resolved. This again implies a priority of responses in homeostasis; e.g., it seems likely that mitigating hypoxia precedes detox cleanup functions.

- Highlight a subset of relevant indicator genes. After documenting which functional sets are activated/deactivated, evaluate which ones would best serve to indicate exposure and stage of impact/recovery in future exposures? Either optimize the currently selected gene panel or create a new microarray monitoring tool?

- The spill data (Figure 1) suggest that transcription activity had not yet baselined by day 83 while TPAH burdens had dropped from 271K to ~3K ng/g (ppb). Are transcription activities still resolving after TPAH levels drop further towards background? Might tracking transcription become a more sensitive method for declaring full recovery or assessing chronic exposures?

- Can transcription profiles distinguish between types of exposures (petrogenic vs other anthropogenic vs natural environmental stressors)?

- Fully developing this approach would provide a monitoring/assessment tool applicable to oil spill researchers and responders throughout subarctic Alaska. Other molluscan species are likely to have similar functional-transcription systems. This project's final gene selection should apply to other molluscs or at least suggest the path to creating a similar tool.

Summary

The complete transcriptomic response to oil exposure would identify the most appropriate genes for monitoring future oil spills and perhaps differentiating between exposure to ANS crude oil and other anthropogenic sources. In addition, by identifying each gene's putative functional role, the proposed study will detail the shifts in activity from one physiological system to another during the recovery process and help elucidate molecular pathways involved during the detoxification process. Connecting the dots between oil exposure's cellular impact and phenotypic or population affects would still be incomplete, but this study would greatly deepen the understanding.

Budget Estimate proposed to PWSRCAC:

Personnel:	Shannon Waters	$8900
	(Lab Manager, will perform all tissue processing and extractions/liaison with UC Davis Genome Center-6 weeks)	
	Lizabeth Bowen	$16,000

	(Principal Investigator, will oversee all laboratory analyses, will work with UC Davis Genome Center Bioinformatics, responsible for all written documents resulting from this study-6 weeks)	
	Brenda Ballachey *(Co-Investigator, will work with Dr. Bowen on interpretation of results and document writing-1-2 week)*	$5000
	James Payne *(Co-Investigator, will work with Dr. Bowen on interpretation of transcriptome/chemistry link-1-2 week)*	$5000
	Bill Driskell *(Co-Investigator, will work with Dr. Bowen on interpretation of transcriptome/chemistry link- 2-3 week)*	$5000
Travel:	no planned travel	$0
Sample collection:	samples have already been collected	$0
Equipment:	no additional equipment required	$0
Supplies:	pre-transcriptome processing *(Pre-transcriptome processing costs only reflect the cost of supplies)*	$17,700
	Transcriptome *(costs for transcriptome analysis include both supplies and analytical costs)*	$17,955
Report writing/presentation:	included in *Personnel* costs	$0
Total:		**$75,555**

References

Carls, M. G., Rice, S. D. & Hose, J. E. Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ. Toxicol. Chem.* **18,** 481–493 (**1999**).

Connon, R. E, Hasenbein, S., Brander, S. M, Poynton, H. C, Holland, E. B, Schlenk, D., et al. (**2019**). Review of and Recommendations for Monitoring Contaminants and their Effects in the San Francisco Bay−Delta. *San Francisco Estuary and Watershed Science*, 17(4). http://dx.doi.org/10.15447/sfews.2019v17iss4art2

Incardona, J. P. *et al.* Cardiac arrhythmia is the primary response of embryonic Pacific herring (Clupea pallasi) exposed to crude oil during weathering. *Environ. Sci. Technol.* **43,** 201–207 (**2009**).

Incardona, J. P.; Gardner, L. D.; Linbo, T. L.; Brown, T. L.; Esbaugh, A. J.; Mager, E. M.; Stieglitz, J. D.; French, B. L.; Labenia, J. S.; Laetz, C. A.; Tagal, M. Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish. *Proc. Natl. Acad. Sci.* **2014**, *111*, E1510–E1518; DOI 10.1073/pnas.1320950111.

Garmendia, L.; Soto, M.; Vicario, U.; Kim, Y.; Cajaraville, M. P. Marigómez, I. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. *J. Environ. Monit.* **2011**, *13*, 915-932; DOI 10.1039/c0em00409j

McKenna, A. M.; Nelson, R. K.; Reddy, C. M.; Savory, J. J.; Kaiser, N. K.; Fitzsimmons, J. E.; Marshall, A. G.; Rodgers, R. P. Expansion of the analytical window for oil spill characterization by ultrahigh resolution mass spectrometry: Beyond gas chromatography. *Environ. Sci. Technol.* **2013**, *47*, 7530-7539; DOI 10.1021/es305284t.

Sørhus, E.; Donald, C. E.; Dasilva, D.; Thorsen, A.; Karlsen, O; Meier, S. Untangling mechanisms of crude oil toxicity: linking gene expression, morphology and PAHs at two developmental stages in a cold-water fish. *Sci. Total Environ.* **2020**, *757*, 143896. DOI 10.1016/j.scitotenv.2020.143896.