



King County

Water and Land Resources Division

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TECHNICAL MEMORANDUM

June 20, 2019

TO: Deb Lester, Supervisor, Toxicology and Contaminant Assessment (TCA), Science and Technical Support Section (STS), Water and Land Resources Division (WLRD), Department of Natural Resources and Parks (DNRP)

FM: Jenée Colton, Water Quality Planner III/Ecotoxicologist, TCA, STS, WLRD, DNRP

RE: 2019 Marine Fish Tissue Contaminant Monitoring Sampling and Analysis Plan Addendum

Introduction

King County will be monitoring English sole (*Parophrys vetulus*) and rockfish (*Sebastes spp.*) for contaminants in King County waters of Puget Sound in June 2019. This memorandum details modifications to the sampling design and analytical methods described in the 2017 Sampling and Analysis Plan (SAP) addendum (King County 2017). Complete sampling and analytical details of the monitoring program can be found in the 2015 Marine Fish Tissue Monitoring SAP (King County 2015).

In response to the West Point Flooding Event in 2017, additional tissue monitoring was conducted to document changes in bioaccumulation of contaminants in several marine species including English sole. This effort was completed as a collaboration between the Washington Department of Fish and Wildlife (WDFW) and King County and included using data from both the WDFW Puget Sound Ecosystem Monitoring Program (PSEMP) and the King County English sole monitoring events. Split samples of English sole tissue collected from Myrtle Edwards and Pier 62 were analyzed by both King County and WDFW. WDFW also sampled two new stations located in the vicinity of the West Point outfall and provided King County split samples to for muscle chemistry analysis. After review of the 2017 data, it was decided response monitoring should continue in 2019. Therefore, to replicate the response monitoring conducted in 2017, King County will collect additional English sole at Myrtle Edwards in 2019 to provide blood, bile, liver, gonad, and muscle tissue samples from individual fish to WDFW. Split composite muscle

samples from West Point North and Pier 62 will also be shared between WDFW and King County for muscle chemistry analysis.

As scheduled, King County has conducted a 5-year review of their freshwater and marine tissue monitoring program designs. The marine tissue monitoring program review included examination of stations, species, and analytes targeted for their effectiveness in meeting the program objectives described in the King County Tissue Monitoring Workplan (King County 2016). This review resulted in design changes that will be implemented in the 2019 monitoring program. In addition, other smaller changes were made to improve the program based on lessons learned in the 2015 and 2017 monitoring events. All changes are described below and will take effect in the 2019 English sole and rockfish monitoring event.

Changes to the 2019 Study Design

Decrease number of English sole in each sample

In 2015 and 2017, six composite samples comprised of 20 English sole each were targeted at each station (120 fish per station or a total of 740 individuals). The time required to process and homogenize all English sole in 2017 was extensive, necessitating approximately 260 hours of labor (note: the target fish total was not reached). This level of staffing and labor were challenging to schedule and consumed roughly 20% of the annual marine monitoring budget. Although WDFW also targets 20 fish per composite, that target is not always met, and some WDFW samples have less fish per composite (e.g., 12-16). To help reduce processing time, King County will target 15 English sole per composite sample in 2019. After the 2019 monitoring event, King County will conduct a power analysis using previous monitoring data to determine how many fish per sample (and how many samples) are actually needed to meet the stated monitoring program objectives.

Eliminate English sole aging

In 2015 and 2017, interopercles were removed from all English sole used for muscle tissue chemistry and analyzed for age by WDFW. Though age data from 2017 are not available at this time, mean age of English sole composite samples in 2015 ranged from 6.2 to 9.4 years. This age range is relatively narrow, particularly in the context of chemical bioaccumulation in fish. On the other hand, rockfish individual ages varied widely from 3 to 32 years old. Thus, to reduce the processing time for the marine monitoring effort, English sole interopercles will not be collected for aging in 2019. Age is highly variable in rockfish and due to their very long lifespan (>), important for interpretation of their tissue chemistry data. Therefore, consistent with prior years, otolith aging analysis will continue for rockfish in 2019.

Eliminate Harbor Island as a monitoring station

In 2015 and 2017, four stations were sampled for English sole and rockfish in Elliott Bay: Alki, Harbor Island, Pier 62, and Myrtle Edwards. Due to the high number of stations and English sole

targeted in this single embayment, we reevaluated the necessity of sampling all four stations to meet the program objectives as listed in King County (2015). As a result, it was decided to eliminate the Harbor Island station in 2019 and continue to target the Alki, Pier 62, and Myrtle Edwards stations.

Mean concentrations of persistent bioaccumulative contaminants (e.g., PCBs and PBDEs) and most metals in English sole from 2015 and 2017 were lowest at the Alki station and higher at the other three stations. The Myrtle Edwards station is most representative of the King County Elliott West combined sewer overflow (CSO) outfall, and the Pier 62 station is most representative of the King Street CSO and areas remediated by King County (e.g., Piers 51-53). Levels of bioaccumulative and most metal contaminants in fish from the Pier 62 station are routinely highest and represent the maximum exposure for fish in Elliott Bay. Conversely, contaminant levels in fish from the Alki station are reliably lowest, except for some metals, making this station representative of minimum exposure for fish in Elliott Bay. Sampling at these three sites will continue in 2019 as they provide data that meet the program objectives.

Although the Harbor Island station is at the mouth of the Duwamish River, close to the East Waterway Superfund Site, specific monitoring stations will be selected within the East Waterway and Lower Duwamish River to document changes resulting from Superfund cleanup actions. The Harbor Island station is not located near any current or historic King County outfalls and no plans for new facilities exist in this area currently. In addition, contaminant levels detected in English sole and rockfish at Harbor Island are generally similar to those in fish either from Myrtle Edwards or Pier 62. Therefore, fish from Harbor Island reflect similar exposure levels as fish from other key locations already sampled. For these reasons, the Harbor Island station will not be sampled in 2019. The potential need to monitor the Harbor Island station can be reconsidered for future monitoring events.

Addition - Pilot of biomarker and PAH-metabolite analyses

The King County Environmental Lab (KCEL) has developed methods for evaluating vitellogenin and polycyclic aromatic hydrocarbon (PAH) metabolites in English sole. Vitellogenin is a protein pre-cursor to egg formation; this protein is found naturally in females but presence in males indicates endocrine disruption. The analytical methods for measuring vitellogenin in blood plasma and PAH-metabolites in bile will be tested in 2019 on a maximum of 30 individual male English sole collected from the Myrtle Edwards and Alki stations (15 fish per station).

On the day of collection, each fish will be measured for length and sexed. Then samples of blood and bile will be extracted from male fish. After dissection, the remaining fish carcass will be frozen and processed for muscle tissue at a later date. Blood and bile collection protocols are described in Attachment 1. The field trawling record sheet (Attachment 2) has been slightly modified from previous years and a new biomarker field datasheet was developed to record information during blood and bile sample collection (Attachment 3).

Vitellogenin analysis of plasma will be completed using the TECO Multi Species Vitellogenin Kit (TE1042) for European flounder, a pleuronectid, validated for English sole, also a pleuronectid. This kit is an enzyme-linked immunosorbent assay (ELISA) test. Antibodies and standards specifically for English sole have been developed by WDFW and the method will be fully validated in spring/summer of 2019.

Additionally, PAHs and their metabolites will be analyzed in the bile of male English sole. Samples will be prepared by dilution and filtration and analysis will involve high-performance liquid chromatography with fluorescence detection (HPLC-FLD). The analysis will target quantification of all PAH metabolites by identifying phenanthrene (PHEN), benzo(a)pyrene (BaP), and naphthalene (NAPH) equivalents. The lower limits of quantitation (LLOQs) for PAH-metabolites are shown in Table 1.

Table 1. PAH Analytes and Lower Limits of Quantitation (LLOQ)

PAH-metabolite	LLOQ (ug/L)
PHEN-equivalents	0.05
BaP-equivalents	0.05
NAPH-equivalents	0.05

Addition - Pilot of per- and polyfluoroalkyl substances (PFAS) analysis

KCEL has developed a method for analysis of PFAS substances. Analysis of PFAS in samples extracted from four frozen northern pikeminnow collected from Lake Union was completed in 2018 with no complications. The method will be further piloted on field-collected English sole muscle and whole rockfish in 2019. Sample preparation includes extraction with sodium hydroxide, drying, and methylene chloride extraction. This is a liquid chromatography (LC) and dual mass spectrometer (MS) (LC-MS/MS) method by isotope dilution. A method Standard Operating Procedure (SOP) is under development. PFAS LLOQs and quality control (QC) limits are listed in Tables 2 and 3. PFAS analysis by KCEL will require the collection of an additional 30 grams of muscle tissue during fish fillet dissection.

Table 2. PFAS Analytes and Lower Limits of Quantitation (LLOQ)

Compound	LLOQ (ug/kg)
L-PFOS	50
PFOA	50

Table 3. QC Limits Table

Parameter	Blank	Matrix Spike	SRM ¹	Surrogates	Spiked Blank
L-PFOS	< LLOQ	20-150	NA	20-150	20-150
PFOA	<LLOQ	20-150	NA	20-150	20-150

¹SRM = Standard Reference Material

Addition of West Point station

WDFW will collect 120 English sole from the West Point North station to replicate the response monitoring for West Point in 2019. WDFW will composite 20 fish in each sample for a total of six samples (120 fish total). WDFW will provide split samples of the composited and homogenized muscle tissue to KCEL for chemistry analysis of the analytes targeted for the other King County muscle tissue samples (King County 2015; excluding PFAS).

Extra Sampling at Myrtle Edwards for West Point Flooding Event Monitoring

As part of the 2019 West Point monitoring activities, WDFW has requested that King County provide tissues from English sole collected at the Myrtle Edwards station for analysis of tissue chemistry and biomarkers. Therefore, KCEL will provide six split samples of composited and homogenized muscle tissue to WDFW for chemistry analysis. In addition, King County will collect an additional 10-30 male fish from Myrtle Edwards and provide extracted blood, bile, liver, and gonad tissues, as well as the corresponding carcasses, to WDFW for analysis of xenoestrogens, PAH-metabolites, vitellogenin, and paired muscle chemistry. Individual fish lengths will be measured then fish will be dissected to identify sex. After sexing, the blood, bile, liver, and gonad tissues will be extracted from male fish within hours of collection. Protocols for blood, bile, liver, and gonad extraction can be found in Attachment 1. However, because WDFW requires 1 to 1.5 mL of blood for their plasma analysis, a 23-gauge syringe will be used to draw blood from an artery along the spine of each fish. This collection method is different from King County's protocol of using capillary tubes to collect blood (Attachment 1). The blood collected for WDFW will be placed in culture tubes containing heparin and chilled until centrifugation to isolate the plasma. Processing of the WDFW blood samples for plasma, and treatment of the final product with aprotinin, will be completed onboard the vessel by a WDFW scientist with a WDFW equipment and supplies.

2019 Monitoring Summary

The 2019 study design is summarized in Table 4. A summary of all targeted analytes for the King County Monitoring Program is shown in Table 5.

Table 4. 2019 Target Sample Numbers by Trawl/Station

Trawl Station	Number of Fish Tissue Samples Targeted				
	Marine Monitoring Program				West Point Event
	Muscle Samples		Whole Body Rockfish Samples	Pilot Biomarkers (Vtg/PAHs/Muscle)	To WDFW for Biomarkers (Vtg/Histo/PAHs)
	English Sole Individuals	English Sole Composite		Male English Sole Individuals	Male English Sole Individuals
West Point N ¹	n/a	6 ¹	--	--	--
Shilshole Bay	90	6	5	--	--
Myrtle Edwards ²	90	6	5	15	10-30
Pier 62 ¹	n/a	6 ¹	5	--	--
Alki	90	6	5	15	--
Quartermaster Harbor	90	6	0	--	--
Totals	360	24 + 12¹ homogenates	20	30	10-30

¹ WDFW will send 6 splits of homogenated composite muscle samples from West Point N and Pier 62 (12 total) to King County for analysis at KCEL.

² KCEL will send 6 splits of homogenated composite muscle samples from Myrtle Edwards to WDFW.

Table 5. Analyte Lists for 2019 English Sole and Rockfish Samples

	English Sole Muscle Composites	Whole Rockfish Individual	English Sole	
			Bile	Plasma
Total Solids	X	X		
Metals + Hg	X	X		
PCBs (H or A)	H	H/A ¹		
PBDEs	X	X		
OC Pesticides	X	X		
PFAS	X	X		
Vitellogenin				X
PAH-metabolites			X	

¹ Rockfish samples will be analyzed by homologue method, but samples with total PCB homologues above 500 ppb will be analyzed for Aroclors as well. In 2017, there were five rockfish with total PCBs above 500 ppb.

H = PCB Homologues

A = PCB Aroclors

Thank you for your attention to this matter. If you have any questions, please do not hesitate to contact me at jenee.colton@kingcounty.gov or 206-477-4075.

cc: Erin McCabe, Water Quality Planner/Project Manager III, King County Environmental Lab

References

King County. 2017. Technical Memorandum: 2017 Marine Fish Tissue Monitoring Sampling and Analysis Plan Addendum. Prepared by Rory O'Rourke and Jenée Colton, Science and Technical Support Section, King County Water and Land Resources Division. Seattle, Washington.

King County. 2016. Tissue Monitoring Work Plan. Prepared by the Toxicology and Contaminant Assessment Unit, Science and Technical Support Section, King County Water and Land Resources Division. Seattle, Washington.

King County. 2015. 2015 Marine Fish Tissue Monitoring Sampling and Analysis Plan. Prepared by Jenée Colton and Chris Magan, Science and Technical Support Section, King County Water and Land Resources Division. Seattle, Washington.

Standard Operating Procedures for Marine Fish Biomarkers – Field Sample Collection

Created May 23, 2019

Specimen Holding

A hose will be used to deliver seawater from the vessel's surrounding waters into the live tank. The seawater pumped into the tank may be continuously flowing and maintained at a specific level using a standpipe drain. Alternatively, the pump could be turned off and the fish could be held in static seawater. Continuous flow through the live tank will be maintained when on station, static seawater (collected en route) will be maintained if fish processing occurs within a harbor/marina, to avoid associated contamination. Adult English sole are an exclusively marine species needing a salinity of 28 ppt or greater to remain in prime condition (WDFW 2016). The equipment used for the live tank will be supplied by WDFW on the vessel.

Decontamination of Tools

Stainless steel tools will be decontaminated using isopropyl alcohol between samples.

Fish and Sample Identification

To begin, several fish will be randomly selected from the live tank and stunned by one or more blows to the head. Each specimen selected for processing will be tagged with a pre-assigned, sequential, unique fish identification number (i.e., Fish ID) that will be created in the planning stage. The assigned Fish ID will then be used for any subsequent blood, bile, and liver samples taken from that fish and will remain with the carcass for resection of its muscle tissues at a later date. Fish will be sexed and only male fish will be processed for blood, liver, and bile samples. Sexing is species-specific, and the methods should be described in the Sampling and Analysis Plan for each project.

Fish ID Tags

Fish identification tags should contain a unique Fish ID number, fish collection date, station name, and fish species. The same unique Fish ID will be applied to all blood, bile, liver, and muscle tissue samples derived from an individual fish. The Fish ID tag will be placed in the storage bag with each fish after it is processed for blood, bile, and liver and before it is processed for muscle tissue samples.

Blood

Blood will be collected from individual fish for later plasma isolation and vitellogenin analysis. After stunning the fish and covering its eyes/head with a paper towel to keep it calm, the

Attachment 1

caudal peduncle will be partially severed with a scalpel blade or knife and blood will be collected from the caudal vein/artery with a heparinized microhematocrit capillary tube. After collection clay plugs will be used to seal the capillary tube. Capillary tubes (single or multiple) with collected blood from each fish will be stored on ice in 15ml plastic centrifuge tubes with caps that have been labeled with the appropriate Fish ID. The tubes will then be transferred to KCEL for immediate centrifugation. Plasma will be isolated by centrifugation for three minutes at 15,000 g within two hours of extraction from the fish. The plasma portion will then be removed from the microhematocrit tube and stored in a centrifuge tube with 0.13 units of aprotinin (a protease inhibitor) at -75° to -85° C until vitellogenin analysis can be completed (Denslow 1999). A minimum volume of 100 uL of blood will be extracted, though additional volume up to 200 uL will be collected if possible. Blood should not be frozen, as this causes lysis of the red blood cells. The plasma portion can be frozen if it will not be further processed on the same day, but note that vitellogenin degradation increases with each freeze/thaw cycle. The holding time for frozen plasma is at least 12 months, while the holding time for plasma at 4° C is 1-2 days (Denslow 1999).

Bile

After extraction of blood, bile samples will be collected from individual fish for PAH metabolite analysis. The gall bladder will be located and secured using clean forceps while a 1-cc 26-gauge tuberculin syringe withdraws the bile. The maximum bile volume will be extracted (up to 1 mL) from the gall bladder and dispensed into a 2 mL amber vial labeled with the appropriate Fish ID. Samples will be stored frozen after collection until transfer to the lab. The holding time for frozen bile is 13 months (Ariese et al. 2005).

Liver

After blood and bile collection, each fish will have its liver removed for CYP1 analysis. Livers or liver pieces up to one gram in weight will be placed in 15 mL centrifuge tubes containing 5 ml of RNA later^{TM} and labeled with the appropriate Fish ID. The liver samples will be stored on ice after processing is completed. Individual samples will remain on ice until transfer to the analytical lab.

Carcass Handling

After blood, bile, and liver samples are removed, the remaining fish carcass will be placed in a Ziploc bag and then double-bagged inside a second Ziploc bag with its Fish ID tag inside the outer bag. The bags with carcasses will be stored in the vessel's on-board freezer until transfer to KCEL. At KCEL the carcasses will be frozen until processed for muscle tissue samples.

Attachment 1

References

- Ariese, F., J. Freek, and D. Beyer. 2005. Two fish bile reference materials certified for PAH metabolites. *Journal of Environmental Monitoring*, 7(9) 869-76.
- Denslow, 1999. Vitellogenin as a Biomarker of Exposure for Estrogen or Estrogen Mimics. *Ecotoxicology* 8, 385-398.
- EPA. 2009. Endocrine Disruptor Screening Program Test Guidelines – OPPTS 890.1350: Fish short-term reproduction assay. Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. EPA 740-C-09-007.
- WDFW. 2016. 2015 English Sole Contaminant Monitoring Survey Post-survey Report. T-BiOS Program, Washington Department of Fish and Wildlife, Olympia, WA.

Attachment 2

Trawl Record for 2019 Marine Fish Tissue Monitoring Event

Date of Collection: _____
Approximate Time: _____
Trawl Location: _____
Vessel: FV Chasina
Trawl Start Coordinates: _____
Trawl End Coordinates: _____

Species	# Taken	Returned		Observations ¹
		Number	Approximate size ranges	

¹ Includes general disposition of species

Notes:

Field Personnel: _____

Attachment 3

Biomarker Sample Datasheet and Chain of Custody (2019)

Sample Date:					Notes: Samples for King County	
Station Name: Myrtle Edwards						
Species: English sole					Whole Body (Y/N)	Comment
FishID	Fish Length (TL, mm)	Sex (F/M/UNK)	Blood (Y/N)	Bile (Y/N)		
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
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23						
24						
25						
26						

Attachment 3

Sample Date:					Notes: Samples for King County	
Station Name: Myrtle Edwards						
Species: English sole					Whole Body (Y/N)	Comment
FishID	Fish Length (TL, mm)	Sex (F/M/UNK)	Blood (Y/N)	Bile (Y/N)		
27						
28						
29						
30						
31						
32						
33						
34						
35						
36						
37						
38						
39						
40						

Chain of Custody - Blood

Relinquished By: _____

Accepted By: _____

Chain of Custody - Bile, Whole Body

Relinquished By: _____

Accepted By: _____