Elliott Bay and Puget Sound Crab Tissue Sampling and Analysis Plan

Final

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King County

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1.0 INTRODUCTION

This sampling and analysis plan (SAP) describes the objectives and the sampling and analysis methods of King County's Elliott Bay and Puget Sound crab tissue monitoring. King County's Marine Tissue Monitoring Program (King County 2016a) includes the collection and analysis of crab tissues every four years. The first monitoring event was conducted in 2014. This SAP is intended to serve the 2018 collection effort as well as future monitoring events.

1.1 Project Background

King County's Marine Tissue Monitoring Program began in 2014 with the collection and analysis of Dungeness and Red rock crab from Elliott Bay and two locations in Central Basin (King County 2014; 2016b). Crab muscle and hepatopancreas samples were analyzed for PCBs (both Aroclors and homologs), metals (arsenic, cadmium, chromium, copper, lead, nickel, selenium, silver, and zinc), and mercury. The 2014 crab sampling enhanced sampling and analysis of Dungeness crab by Washington Department of Fish and Wildlife (WDFW) conducted throughout Puget Sound in 2011 and 2012 (Carey et al. 2014). Crab muscle and hepatopancreas samples collected by WDFW were analyzed for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, five metals (arsenic, cadmium, copper, lead and zinc) and mercury.

As outlined in County's Tissue Monitoring Work Plan (King County 2016a), crab will be monitored every four years. The goals of the Tissue Monitoring Program are included in the Work Plan with key goals for crab monitoring highlighted below.

Crab samples will be used to monitor contaminant concentrations in Dungeness and Red rock crab caught near public fishing piers or public access points. Both crab species are consumed by recreational and tribal fishers, and thus, these data can be used by public health agencies to update any consumption advisories, as necessary, as well as provide a general comparison of contaminants in crab collected from the Lower Duwamish Waterway and East Waterway Superfund sites. In addition, these data can be used to estimate of both ecological and human health risks and to assist in documenting the benefits and effectiveness of water and sediment quality investments over time.

1.2 Scope of Work

The crab species targeted are Dungeness (*Metacarcinus magister*) and Red rock (*Cancer productus*). Crab will be collected near public access points at five locations in central Puget Sound: Shilshole, Elliott Bay, West Seattle, Redondo, and Vashon Island.[1] Both crab species will be targeted at each location. However, both species may not be obtained at each location because suitable habitats for both species may not be present. For example, hard substrate is a preferred habitat for Red rock crab while sand is preferred habitat for

[1] New locations have been added on Vashon Island since the 2014 sampling.

Dungeness crab. Crab muscle and hepatopancreas tissue samples will be analyzed for select bioaccumulative chemicals including PCBs, PBDEs, metals and mercury, organochlorine pesticides, as well as lipid and total solids content. The targeted analytes are discussed in more detail in Section 2.2.2.

1.3 Schedule

Crab will be collected in late April and May 2018. Future monitoring years will also target late spring. Sampling does not need to occur on consecutive days and can be scheduled to optimize collection efficiency and accommodate staff availability. The field effort is anticipated to last up to four days.

The King County Environmental Laboratory (KCEL) will analyze all crab tissue samples. The turn-around time for analytical data is approximately eight to ten weeks from the date of last sample receipt, at which time entry of quality assurance (QA)-approved data into the laboratory information management system (LIMS) should be complete. A data report will be prepared after all analytical data has been assessed and a QA review completed. The data report is anticipated to be completed within six to nine months of final posting of analytical results in LIMS.

1.4 Project Team

The following King County staff members are responsible for project execution:

Jenée Colton, Water and Land Resources Division (WLRD) Marine Tissue Monitoring Program Manager 206-477-4075

Debra Williston, WLRD Project Manager..... 206-477-4850

Rory O'Rourke, WLRD SAP Development..... 206-477-4715

Fritz Grothkopp, KCEL Project Manager..... 206-477-7114

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Bob Kruger, KCEL Field Science Unit Lead..... 206-477-7147

2.0 SAMPLING DESIGN

The following sections provide a description of the study design including data quality objectives (DQOs), sampling methods, and field and analytical parameters.

2.1 Data Quality Objectives

The project manager will assess project data and evaluate whether the data collected are of sufficient quality to meet monitoring goals. The DQOs of precision, accuracy, bias, representativeness, completeness, comparability, and sensitivity are described in the following sections, along with data assessment. Data not meeting objectives will be identified and potentially used to improve future monitoring efforts.

2.1.1 Precision, Accuracy, and Bias

Precision is the agreement of a set of results among themselves and is a measure of the ability to reproduce a result. Accuracy is an estimate of the difference between the true value and the measured value. The accuracy of a result is affected by both systematic and random errors. Bias is a measure of the difference, due to a systematic factor, between an analytical result and the true value of an analyte. Precision, accuracy, and bias for analytical chemistry may be measured by one or more analyses of various laboratory QC samples such as blanks, surrogates, and replicates.

Field collected tissue samples are expected to be fairly variable between samples and mostly driven by size and species. Accuracy will be assessed using laboratory spike or laboratory control samples. Precision will be evaluated by the use of laboratory duplicate samples.

Analytical bias cannot be quantified for tissue analysis, particularly for PCB homologs, which are mixtures of different PCB congeners. However, the composite crab samples will target average concentration and reduce some of the natural variability between individual crabs. Standard Reference Materials (SRMs) will be used whenever available to better quantify specific analytical method accuracy and precision.

2.1.2 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point, or an environmental condition. The species and size of crab targeted for collection were selected so general comparisons to past collection efforts can be made. Following the guidelines described in Section 3 for sample collection, processing, and handling will also help ensure that samples are representative.

2.1.3 Completeness

Completeness is defined as the total number of samples analyzed for which acceptable analytical data are generated, compared to the total number of samples submitted for analysis. Sampling with adherence to standardized sampling and testing protocols will aid

in providing a complete set of data for this survey. The goal for completeness is 100%. If 100% completeness is not achieved, the project team will evaluate if the DQOs can still be met or if additional sample collection and analysis is necessary prior to data reporting and analysis.

2.1.4 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal is achieved through using standard techniques to collect and analyze representative samples, along with standardized data assessment and reporting procedures. By following the guidance of this SAP, the goal of comparability between this, past and future sampling events will be achieved. Use of established techniques enhances regional comparability.

2.1.5 Sensitivity

Sensitivity is a measure of the capability of analytical methods to meet the monitoring goals. The analytical detection limits presented in Section 4.0 are sensitive enough to detect analytes at concentrations of interest to assess significant changes in crab tissues.

2.1.6 Data Assessment

Chemical data and quality control (QC) results will be assessed against requirements of the analytical methods as well as the requirements of this SAP.

2.2 Sampling and Analytical Strategy

Crab will be collected using pots deployed by boat. Target sample numbers are listed in Table 1. Each sampling location will target 15 Dungeness and 15 Red rock crabs. The details on sizes and sex of crab as well as number of pots per sampling location are provided in Section 3.1.

Table 1. Target species, sizes, and sample numbers.

Species	Tissue type	Target number of composites	Target number of crab per composite	Total Crab
Dungeness Crab	Muscle	25	3	Up to 75
Red Rock Crab	Muscle	25	3	Up to 75
Dungeness Crab	Hepatopancreas	15	5	Dissected from whole
Red Rock Crab	Hepatopancreas	15	5	Dissected from whole
	Total	**80**	**---**	Up to 130 combined of both species

2.2.1 Sampling Station Locations and Sample Identification

Crab will be collected from five general locations: Shilshole, Elliott Bay-Seattle waterfront (Terminal 86 Pier), Elliott Bay-West Seattle (Seacrest Pier and/or Duwamish Head), Redondo, and Vashon Island (Quartermaster Harbor and/ or Tramp Harbor) (see Figure 1). These locations are similar to the previous collection effort by King County in 2014 with the addition of the Vashon Island locations.

A paired location indicates that 15 Dungeness and 15 Red rock crabs will be acquired from the combination of catch from both stations. These stations are paired either due to historically low catch (e.g. Seacrest Park Pier) or one of the stations having habitat that is expected to support only one species (e.g. Quartermaster Harbor). The following approach is used for each of the paired locations:

- Due to low catch at Seacrest Park Pier location in 2014, sampling at this location will depend on catch success after the first pot deployment. If catch is low (fewer than 3 legal crabs cumulatively from pots deployed during initial soak) at this location, then efforts can be abandoned at Seacrest Park and moved to nearby Duwamish Head station. Seacrest Park may prove to be successful for one of the targeted crab species. In this case, Duwamish Head will be targeted for the crab species not obtained at Seacrest Park.
- At Vashon Island, both Quartermaster Harbor and Tramp Harbor are being sampled. A WDFW standard test fishery site for Dungeness crab is by the entrance of Quartermaster Harbor in the Manzanita-Rosehilla area of Vashon Island (Velasquez and Rothaus Pers. Comm. 2018). Based on information from WDFW, it is expected that Quartermaster Harbor location will not have Red rock crab due to lack of preferred habitat for this species. Therefore, Quartermaster Harbor location is being targeted only for Dungeness crab. WDFW suggested Tramp Harbor as a good alternative for targeting Red rock crab. Therefore, both Quartermaster Harbor and Tramp Harbor are being sampled to increase the success of collecting Dungeness and Red rock crabs at Vashon lsland. If at least 3 legal crab cumulatively from pots deployed during the initial soak at Quartermaster Harbor are not obtained, then efforts can be abandoned at this location and efforts will be focused at Tramp Harbor, which is expected to have habitats preferred by both crab species.

It is possible that each crab species could be collected from both paired locations to reach the target of 15 crabs of each species. In this case, crab will be retained if at least groups of three crabs can be collected (relates to number of crab per muscle composite sample). The project manager will be consulted after the initial soak at paired locations to discuss the best approach based on numbers of crabs present in pots.

A Locator Identification code generated for each of the seven potential sampling locations is shown in Table 2. The specific coordinates of each crab pot deployment location will be recorded by KCEL Field Science Unit (FSU) staff on field sheets (see Appendix A).

Table 2. Locator ID and general coordinates for each sampling location.

Location	Locator ID	Paired Location	Easting[a]	Northing[a]
Shilshole Bay Marina	CB-SHMarina-N	N	1253476	254630
Terminal 86 Pier	EB-T86Pier	N	1260471	232101
Duwamish Head	EB-DuwHead	Y	1256857	221836
Seacrest Park Pier	EB-SCPPier		1258804	218982
Tramp Harbor	CB-TrmpHrbr	Y	1243638	154737
Quartermaster Harbor	CB- Qrtrmster		1232343	131654
Redondo Pier	CB-Redondo	N	1270333	130936

[a] Coordinates represent general sampling locations. Coordinates are in State Plane North NAD83 US Survey Feet.
Y = Yes
N = No

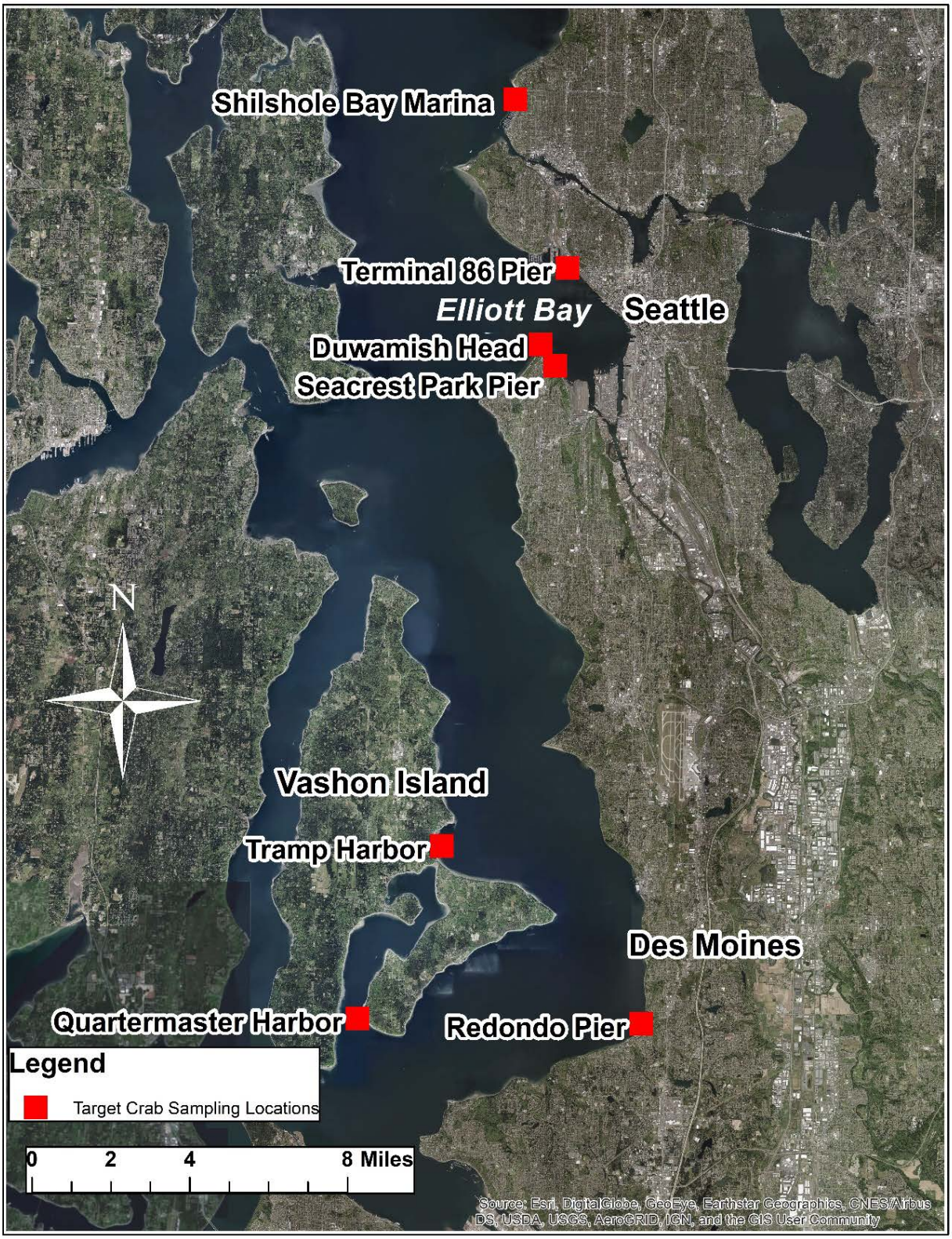

Figure 1. Dungeness and Red rock sampling locations.

2.2.2 Sample Acquisition and Analytical Parameters

Sampling will be conducted using commercially available recreational crab pots. Sampling will be conducted by KCEL FSU staff. Samples in 2018 will be collected under Permit EASH-LOUCKS 18-095 issued by WDFW (Appendix B). Future sampling will be conducted under the WDFW collection permit obtained for that year's sampling event.

Each sample will be analyzed for total metals (arsenic, beryllium, cadmium, chromium, copper, lead, nickel, selenium, silver, thallium, and zinc), mercury, PCB homologs, PBDEs, organochlorine pesticides, total solids, and lipids. The organochlorine pesticides may be discontinued in future monitoring efforts if these parameters are largely undetected. All analyses will be conducted by KCEL. If constraints, such as sample mass, limit analyses, the following prioritization will be followed:

1. PCB homologs
2. Total Solids
3. Metals
4. Mercury
5. Total lipids
6. PBDEs
7. Organochlorine pesticides

The minimum tissue mass required for each analysis type is presented in Table 3.

Table 3. Minimum analytical mass requirements for tissue samples by matrix

Analyte	Muscle Tissue / Hepatopancreas	Extra mass for Quality Control Analyses
PCB Homologs	20 g / 10 g	80 g for muscle; 40 g for hepatopancreas
Metals	1.25 g / 1.25 g	2.5 g for muscle; 2.5 g for hepatopancreas
Mercury	1 g / 1 g	2 g for muscle; 2 g for hepatopancreas
PBDEs	25 g / 15 g	80 g for muscle; 40 g for hepatopancreas
Chlorinated Pesticides	NA	Co-extracted with PBDEs
Total Solids	1 g / 1 g	1 g for muscle; 1 g for hepatopancreas
Lipids	NA	Co-extracted with PBDEs
Total Mass needed	**48.25 g / 28.25 g**	**250.5 g for muscle; 125.5 g for hepatopancreas**

3.0 SAMPLING PROCEDURES

This section describes the field collection methods and sample processing procedures to be followed to meet the monitoring DQOs: representativeness, comparability, and completeness. Sample handling, storage, and preparation will generally follow EPA (2000) guidance.

3.1 Sample Collection

The field lead will ensure that a copy of the collection permit from WDFW is with the sampling crew and crab pots are appropriately labeled. Crab will be collected using large recreational or commercial crab pots deployed from a boat. Crab pots will be baited with fish (either heads/tails or herring), squid, cockles, geoduck, and/or poultry necks/legs. Crab bait will be placed in mesh bait bags or boxes and tied to the inside of the trap so the bag cannot be opened and its contents consumed. Escape rings may be closed off with wire to prevent escape of crabs (Velasquez and Rothaus Pers. Comm. 2018). Pots will be deployed in locations to avoid ship, boat, and other vessel traffic.

Locations of individual pots can affect catch success of Dungeness and Red rock crabs even within a sampling location due to varying habitat characteristics. As such, at least three pots will be deployed along a gradient from shallower to deeper water at each general sample location for the first pot deployment. The first pot deployment locations were based catch records from previous sampling and knowledge shared by WDFW staff. A description of pot targets are included in Table 4.

Table 4. First deployment pot targets.

Pot Number by Sampling Location	Depth (-ft MLLW)	Description
Shilshole Bay Marina[a]		
#1	20 - 30	Inside breakwater at S entrance to marina
#2	10 - 20	Inside breakwater at N entrance to marina
#3	50 - 60	Outside breakwater between N and S marina entrances
Terminal 86		
#1	30 - 40	Adjacent to fishing pier's obstruction fish haven on W or E; adjust based on degree of bottom slope
#2	50 - 60	~ S of fishing pier, perpendicular to shore
#3	80 - 90	~ S of fishing pier, perpendicular to shore

Pot Number by Sampling Location	Depth (-ft MLLW)	Description
Duwamish Head		
#1	12 - 15	~ due N of fishing pier
#2	20 – 30	~ W/SW of Pot #1
#3	50 - 70	~ E of Pot #1
Seacrest Park Pier		
#1	30 – 40	NW of pier
#2	60 - 70	N/NE of pier, perpendicular to shore
#3	100 - 110	NE of pier, perpendicular to shore
Tramp Harbor		
#1	20 – 25	~ NNE of fishing pier
#2	60 - 80	~ NE of Pot #1
#3	30 - 50	Adjacent to W corner of Point Heyer/ Ellisport obstruction fish haven
Quartermaster Harbor		
#1	35 - 45	Due N of red can at harbor entrance
#2	55 - 65	Due S of red can at harbor entrance
#3	80 - 90	~ SW of Pot #2
Redondo Pier		
#1	50 – 60	~ NW of fishing pier, perpendicular to shore
#2	75 – 95	~ NW of Pot #1, perpendicular to shore
#3	110 - 120	~ NW of Pot #2, perpendicular to shore

ft MLLW = feet mean lower low water

Based on field conditions, these locations and depths may be adjusted.

[a] Attempts to collect Dungeness crab from inside the breakwater were first attempted in a separate County monitoring program in May 2017 but only Red rock crabs were collected; pot deployment was moved to off-shore of the breakwater on advice from WDFW where Dungeness crab collection was successful.

This first pot deployment will be for an "initial soak" of approximately three to five hours[2]. Field staff will re-deploy pots a second time at the location depths from the initial soak with the greatest number of each crab species catch. During this second soak, crab pots will be deployed for approximately 12–20 hrs (overnight) and then retrieved. A target of 15 male Dungeness and 15 male or female Red rock crabs will be collected at each location or paired location. Sampling is expected to occur over a maximum of four days. If 15 crabs of each species are collected at a location within a shorter period, no further collections will occur at that location and these additional pots maybe deployed at other locations to increase the probability of collecting the target number of crabs. An additional pot deployment for a third soak for approximately 3-5 hours will occur if the target number of crabs have not been collected at a particular location and the maximum field effort has not been reached. The project manager will be consulted to confirm if this third soak is warranted.

Legal sized crab will be targeted, thus carapace size should be at least 6.25" for male Dungeness and 5" for male and female Red rock. However if only undersized crab are being caught, crab within ½ inch of legal size will be retained. Crab carapace width measurements will be made in the field using crab gauge (available at marine supply stores) to ensure appropriate size is being retained. In keeping with WDFW guidance, crab carapace width measurements will be made laterally across the carapace from just inside points. Excess or undersized crab, non-target crab species, softshell crab, or female Dungeness crab will be returned to the water immediately at their location of capture. The types and number of species caught in the crab pots will be recorded on field record sheets for each location (see Appendix A). The size range will be estimated for those crabs under the legal size. For those crab retained for analysis, the field sheets will record the species, number, collection date, pot number and pot coordinates.

The pot number will be a simple letter and number sequence. Pots will be labeled A, B, C, D, etc., and then simple number sequence added for each pot deployment. For example, at Shilshole, the first pot deployed will be Pot A-1, the second pot deployed will be Pot B-1. If these pots are deployed on another day at this location, they would be pot A-2 and B-2. If a pot from one location is deployed at another location, the letter designation will remain and the pot number sequence will be the next one available. For example, a pot that had been used twice at Shilshole is used at Terminal 86 Pier, the pot would be labeled A-3 (this states this is the 3rd deployment for this particular pot). Because the locator and pot coordinates are on the collection form, the location the pot is deployed will be known.

Field staff will wear protective thick vinyl or plastic gloves for personal protection from crab claws. Also, because the target tissue for this study is muscle and hepatopancreas, the shell and shell membranes of undamaged specimens afford protection from potential environmental contaminants during the retrieval from the pot. For these reasons, field staff will not be required to wear nitrile gloves. To optimize the natural shell and membrane protection, specimens with damaged shells will not be accepted as samples.

[2] In 2014 SAP, pots were deployed for overnight soak. The change to an initial four hour soak was suggested by WDFW crab biologists to maximize catch efficiency (Velasquez and Rothaus Pers. Comm. 2018).

Target crab specimens will be measured with crab gauge in the field to ensure target size is retained; detailed measurements of size and weight of retained crab will be conducted at KCEL. Acceptable crabs are bagged and placed on ice in coolers until they can be frozen, which will be no more than 12 hours after collection. Individual specimens from a particular pot will be kept together in one large resealable plastic bag(s) with the date, time, locator, pot number, and species recorded on the outside in indelible ink (see Section 3.8). All other pertinent information will be traceable through the field sheets or field notebook. The bagged and iced crabs will be transported in coolers to KCEL on the same day they were collected.

3.2 Softshell Crabs Identification and Handling

Only hardshell crabs will be retained for chemistry samples. The sampling timeframe is expected to overlap with the end of molting season in Marine Areas 10 and 11; therefore field staff may encounter softshell crabs (Velasquez and Rothaus Pers. Comm. 2018). Softshell crabs have lost their hard exoskeleton during molting, a normal part of the crab's growth process. However, losing this hard exoskeleton shell can also make crabs significantly more susceptible to unintended mortality, even during careful field handling (Kruse et al. 1994). Included are steps for identifying and handling softshell crabs to reduce mortality (https://wdfw.wa.gov/fishing/shellfish/crab/softshell.html). Crab of target collection size will be treated as follows per the WDFW guidelines:

1. Grab it from the back and turn it upside down (ventral side facing up).
2. Carefully push the "elbow" of one claw towards the mouth of the crab, exposing the shell that is usually covered by the folded claw (Figure 2).

Figure 2. Pushing "elbow" of the claw towards the mouth of the crab.

3. Pinch the shell with your thumb at the point shown, gradually increasing the pressure (Figure 3). Remember, you do not want to break the shell while performing the test (crabs have blood and can bleed to death). If the shell flexes or

bends, release the pressure and return the crab carefully to the water. Take care not to drop any crab which you release onto hard surfaces and do not fling them roughly into the water. If the shell has not flexed and you have reached the point at which you would have easily crushed a tough peanut, then you have a hard (and legal) crab to keep.

Figure 3. Pinching shell at point shown.

4. Experienced crabbers note that there are other indicators that crabs may be soft-shell. While these indicators are not the definitive test used in Washington, they do help people quickly identify crab which are likely to be soft-shell. Some pinch the center of the large section of the first walking leg in a test similar to the one above (Figure 4).
5. In most cases, the two pinch tests yield identical results, however some crab will pass the former test while failing the leg test. After lifting many legal size crab, people note that the crab which seem light for their size are generally soft-shell. The shells of soft-shell crabs also tend to be white on the underside, while those of hard-shell crabs are a darker yellowish brown (Figure 5) and often covered with barnacles and algae.

This is intended to be a basic set of procedures for determining shell condition. More detail is provided in WDFW's Dungeness Crab Shell Condition Field Manual (Lippert et al. 2002).

Figure 4. Demonstrating the walking leg pinch test.

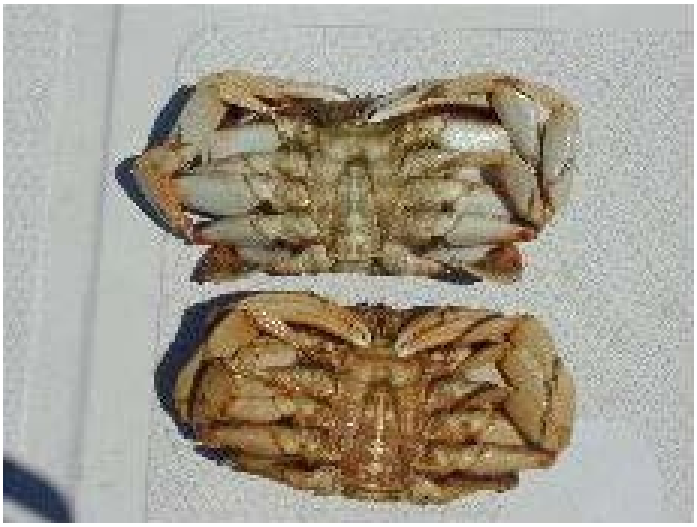

Figure 5. Demonstrating color differences between softshell (top) and hardshell (bottom) Dungeness crabs.

3.3 Field Sampling Equipment

Field items needed are identified below. The field lead will check that all equipment is included and in working order each day before sampling personnel go in the field.

1) Crab Pots
    a) Pot with lines, floats, weights
    b) Bait
    c) Crab Pot labels with King County name and address
    d) Wire (to close escape rings)

2) Sampling supplies:
    a) Coolers
    b) Ice
    c) Gallon size and 2-gallon size freezer plastic bags
    d) Pens and markers with indelible ink
    e) Crab gauge
    f) Global positioning system device
    g) Nitrile gloves (optional)
3) Safety equipment:
    a) Personal floatation device
    b) Thick vinyl or plastic gloves for personal protection
    c) Rain gear
    d) Cellular phone
    e) First aid kit
4) Documentation supplies:
    a) Field notebook and field sheets (with Chain-of-Custody stamp)
    b) Sample labels
    c) Camera

3.4 Sample Processing

The following describes the steps for sample processing and homogenizing of crab samples. Dissection and homogenization procedures follow the recommendations in EPA (2000). Homogenization of composite samples will not occur until project manager has been consulted on the final compositing scheme, which will depend on the numbers and species of crab collected.

At the end of each sampling day, the iced crabs will be brought to KCEL for initial processing. Immediately upon return from the field, weighing and measuring of the crab will occur by FSU staff. Crab carapace width measurements will be obtained using stainless-steel calipers. In keeping with WFDW guidance, crab carapace width measurements will be made laterally across the carapace from just inside points (Figure 6). Crabs will be weighed using a laboratory balance suited for the weight of the species. Crab will be identified to species, measured to the nearest 1 mm, and weighed to nearest 0.5 g; this information will be recorded on sample processing sheet (see Appendix A). Crabs unique to a location and pot deployment will be recorded on individual processing sheets. Final processing steps, which include dissection of muscle meat and hepatopancreas, homogenization of tissues, and packaging tissue homogenates samples for freezer storage, will occur following completion of all crab collection efforts.

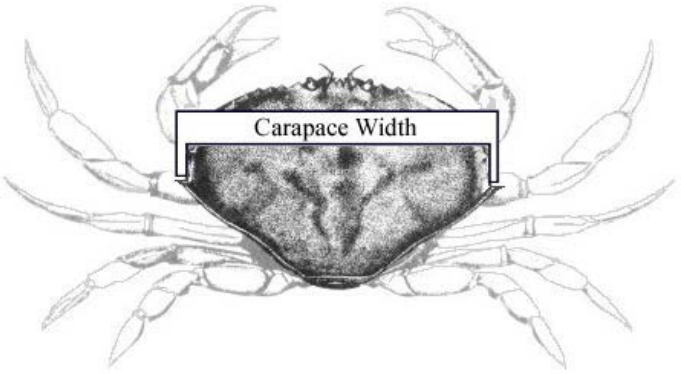

Figure 6. Location of crab carapace width measurements

In general, composite samples will comprise individual crabs of different carapace size such that average size is similar between composite samples. Composites will be composed of three crabs per muscle tissue sample and five crabs per hepatopancreas tissue sample, with each crab supplying approximately equal mass of muscle or hepatopancreas tissue respectively. The final compositing scheme will be determined in consultation with the project manager after completion of all collection efforts. Both muscle and hepatopancreas tissues will be dissected from the crab using cleaned stainless steel implements. Muscle tissues will be homogenized with a Tissumizer®, while hepatopancreas will be homogenized in the sample jars using a spatula. Each composite should be comprised of approximately 200-300 g of muscle tissue and 150 g of hepatopancreas tissue. As noted in Table 3, the minimum amount is 48.25 g of muscle tissue and 28.25 g of hepatopancreas but for samples targeted for QA analyses at least 250 g of muscle and 150 g of hepatopancreas tissues are needed. Sample jars will be labeled and placed in freezer storage (see Section 3.5). Composited crab will be recorded on the Sample Compositing Sheet (see Appendix A). This sheet will include recording of mass aliquot including in the composite sample along with information from the sampling processing sheet in order to track the size and species of crab tissue per composite.

Final processing of samples will follow these general steps:

Frozen crab will be thawed enough to allow processing.

1) Knives and sample processing tools (forks, hemostats, pliers) will be detergent washed, reverse osmosis (RO) water rinsed, propanol rinsed, and air dried. Knives and other instruments may be ceramic, stainless steel, or PTFE (Teflon). The same clean instruments/surface can be used repeatedly, without re-cleaning, on specimens contributing to the same composite; however, different pre-cleaned tools will be used between hepatopancreas and muscle tissues. All processing equipment must be subjected to the complete cleaning procedure between composites. Lab personnel will wear nitrile gloves that must be changed between composite samples. A “clean” work-surface, means a surface (lab counter, polypropylene cutting board, sorting tray, etc.) covered by aluminum foil fresh off

the roll. The work surface is covered with at least one layer of aluminum foil and the foil must be changed between composites.

2) Working on a clean work surface per step 2 above, the abdomen of each crab will be removed to access the hepatopancreas, which will be removed first and placed directly into a labeled sample jar. The hepatopancreas also known as "crab butter" is observable as a greenish to brownish paired organ. It can be removed while the crab is still slightly frozen or thawed; avoiding significant mixing of body fluids, or loss of tissue if it becomes fluid.
3) The remainder of the crab body will be dissected using a knife and forceps while the claws will be separated from the body. Muscle tissue for the composite should be taken from the body, legs, and the claws. A hemostat or tweezers may be used to remove muscle from the claws or cleaned pliers may be used to break them open. Once removed, muscle tissue will be placed into labeled sample jar. Muscle tissue that was in contact with hepatopancreas, should not be included in the sample.
4) Individual crab muscle tissue will be homogenized in sample jar with a Tissumizer. Individual crab hepatopancreas tissue will be homogenized in sample jar with a spatula.
5) After all crab in the composite sample are dissected and tissue homogenized, an equal mass aliquot of muscle tissue from each crab will be transferred to a new sample jar and homogenized with a Tissumizer. This step is repeated in separate container for hepatopancreas tissue using a spatula.
6) Clean cutting board, dissection tools, and the blender as noted in Step 2 between composite samples.
7) Completed homogenized samples will be stored frozen until analysis (see Section 3.5). Prior to analysis, tissue will be thawed and sufficient mass (either muscle or hepatopancreas) from the jar will be removed for analysis; extra homogenate tissue will be archived.

3.5 Sample Delivery and Storage

Crab will be held in labeled plastic bags on ice in the field. At the end of each sampling day, all crabs will be transported back to the KCEL, initially processed as described in Section 3.4 and then stored frozen at KCEL until final processing. Final processing of crab will occur within approximately 10 days of last collection day. After all processing is complete, the holding times in Table 5 apply to the composite samples.

Table 5. Sample containers, storage conditions, and analytical hold times

Analyte	Container	Preferred Storage Conditions	Hold Time	Acceptable Storage Conditions	Hold Time
Total Solids	4-oz. CWM PP container	freeze at ≤-18°C	6 months to analyze	refrigerate at 4°C	14 days to analyze
Mercury	4-oz. CWM PP container	freeze at ≤-18°C	28 days to analyze	refrigerate at 4°C	28 days to analyze
Total metals	4-oz. CWM PP container	freeze at ≤-18°C	2 years to analyze	refrigerate at 4°C	180 days to analyze
PCB Homologs	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
Lipids	16-oz. glass	freeze at ≤-18°C	1 year to extract Analyze immediately after extraction	refrigerate at 4°C	14 days to extract Analyze immediately after extraction
PBDEs	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
Chlorinated Pesticides	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze

CWM PP – Clear, wide-mouth polypropylene

3.6 Chain of Custody

Chain of custody (COC) will commence at the time that each crab pot catch is collected. All samples will be under direct possession and control of King County field staff. All sample information will be recorded on a COC form which is the same as the field record sheet (Appendix A) with a KCEL stamp. This form will be completed in the field and will accompany all samples during transport and delivery to KCEL. Upon arrival at the KCEL,

the samples will be relinquished to sample login. The date and time of sample delivery will be recorded and both parties will then sign off in the appropriate sections on the COC form at this time. Once completed, original COC forms will be archived in the project file.

3.7 Field Replicates and Equipment Blanks

Field replicates are intended to show within station variability such as may exist at a sediment or water sampling station. For tissue samples, a true field replicate cannot be collected because each organism at a station is inherently different (e.g., age, size, diet) so no individuals would be expected to have exactly the same tissue chemistry. Collection and analysis of trip blanks or equipment blanks from crabbing equipment is neither practical nor necessary for this project. The amount of contamination from equipment or sample exposure during transport to the laboratory is relatively insignificant compared to the concentrations measured in tissue. Therefore, not field replicates or equipment blank samples will be collected.

3.8 Sample Documentation

Sampling information and sample metadata will be documented using the methods noted below.

- Field sheets and field notebooks will be used at all stations to record the following information:
    1. station name (locator)
    2. crab species collected
    3. coordinates of each crab pot per deployment, crab pot number, and counts of how many of each of the targeted species were retained from each pot deployment
    4. date and time of sample collection
    5. approximate water depth
    6. counts of species and approximate size of each returned to the water
    7. notes on condition of crab retained
    8. deviations from sampling procedures
    9. unusual conditions (e.g., water color or turbidity, presence of oil sheen, odors)
    10. names of all sampling personnel
- Crab processing sheets will be completed recording station name (locator), pot number, species, lengths, and weights of individuals, and sex of individuals. Sample compositing sheets will be completed recording station name (locator), species, tissue type, LIMS Sample ID, and sample mass aliquot, and number of individuals from which the composite was derived (see Appendix A for details).

- LIMS-generated container labels will identify each container with a unique sample number, station and site names, collect date, analyses required, and preservation method. Note the same sample ID will be used for all analyses for that sample.
- COC documentation will consist of the KCEL standard COC stamp on the field record sheet (Appendix A), which is used to track release and receipt of each sample from collection to arrival at the lab.

4.0 ANALYTICAL PARAMETERS AND METHODS

Analytical methods and associated detection limit goals and quality control requirements for the analyses performed are presented in this section. In future sampling events, updates to methods or detection limit goals will be documented in a SAP addendum.

The detection limit goals for metals, total mercury, PCBs, PBDEs and chlorinated pesticides are based on the Lower Limit of Quantitation (LLOQ)[3] while those for total solids and lipids are based on method detection limit (MDL) and reporting detection limit (RDL). Both LLOQ and RDLs are considered analogous to a Practical Quantitation Limit.

The LLOQ can be no lower than the lowest concentration on the calibration curve and must be verified according to the requirements in each reference method. For SW-846 methods, concentrations less than the LLOQ will be qualified in the KCEL LIMS with a "<QL" flag. The listed LLOQs can change in any given sample due to matrix interference, sample dilutions and/or sample weight extracted, and thus, sample LLOQs may differ from the goals listed in this SAP. In addition, LLOQs are periodically evaluated and may increase or decrease. Every effort will be made to meet the quantitation limit goals listed in this SAP. Any changes to the instrument LLOQ values will be documented in a Data Anomaly Form or project narrative as a deviation from the SAP.

For metals (Methods 6020B), mercury (7471B) and chlorinated pesticides (Method 8081B), sample results may not be reported below the LLOQ and, therefore, the LLOQ value for each parameter is entered into both MDL and RDL fields in LIMS. Sample results for PCB Homologs and PBDE Congeners may be reported at concentrations below the LLOQ if the qualitative results for the parameter meet the requirements of the reference method. In LIMS, the LLOQs will be reported in the LIMS RDL field, while the MDL will represent the minimum concentration where the qualitative criteria for the method can be met (typically one-half the LLOQ). Numeric values reported between the LLOQ and the limit of qualitative method criteria will be flagged "<QL, J", since they are considered estimates.

For lipid and total solids analyses, which are not based on SW-846 reference, the sensitivity limit is still defined as the MDL, which is calculated using the procedure in 40 CFR Part 136, Appendix B. The value determined by this procedure may be increased to account for method variability, and will be reported. The RDL is calculated by multiplying the MDL by a factor between 2 and 10, depending on the parameter. For methods where the MDL is applied, parameters less than the MDL will be qualified with a "<MDL" flag. Sample MDLs and RDLs may differ from the target detection limit goals as a result of necessary analytical dilutions or a reduction of extracted sample amounts based upon available sample volumes. Every effort will be made to meet the detection limit goals listed in the SAP.

[3] The EPA analytical reference methods used for this project belong mostly to the SW-846 compendium of analytical methods and require that sensitivity be defined as the "lower limit of quantitation" (LLOQ) and no longer require MDL studies.

4.1 Metals

Metals will be analyzed by inductively coupled plasma mass spectrometer (ICP-MS) according to KCEL SOP # 623. This method generally follows PSEP (1997) and EPA SW-846 6020B protocols. This SW-846 method does not allow data to be reported below the LLOQ. Tissue samples require acid digestion before analysis. LLOQs for ICP-MS metals are based on an initial sample weight of 1.25 (± 0.05) g and a final volume of 50 ml. The targeted metals and associated detection limit goals are summarized in Table 6.

Table 6. Metals target analytes and detection limit goals

Analyte	LLOQ (mg/kg ww)
Antimony	0.012
Arsenic	0.002
Barium	0.02
Cadmium	0.002
Chromium	0.008
Copper	0.008
Molybdenum	0.004
Nickel	0.004
Lead	0.004
Selenium	0.02
Thallium	0.004
Vanadium	0.003
Zinc	0.02

LLOQ = Lower Limit of Quantitation.
Method blanks will be evaluated down to a value that is one-half the LLOQ.

4.2 Mercury

Total mercury will be analyzed according to KCEL SOP # 604 using cold vapor atomic absorption spectrometry (CVAA). The analysis is reported under PSEP (1997), which retains method elements of EPA 245.1 revision 3, SW-846 7470 and 7471B. Tissue samples require acid digestion before analysis. LLOQs for mercury are based on an initial sample weight of 0.667 (±0.05) g and a final volume of 100 ml. The detection limits targeted are shown in Table 7.

Table 7. Mercury detection limit goals

Analyte / Range	LLOQ (mg/kg ww)
Mercury (Low Range)	0.00038
Mercury (Mid-Range)	0.004

LLOQ = Lower Limit of Quantitation.

4.3 PCBs

PCB homolog analysis will follow KCEL draft SOP #782, which generally follows the guidelines of EPA methods 680 and 1668C. This method relies on the quantitation of congeners using gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM). This low-resolution method will generate PCB concentrations based on each of the 10 homolog groups, which can then be used to generate a total PCB concentration. Sample preparation is described in KCEL SOP# 705 for soils, tissues, and sediments. The preparation method is a soxhlet technique following EPA method SW-846-3540C using methylene chloride as the extraction solvent. The cleanup method is based on Method 1668C with gel permeation cleanup (GPC) followed by an anthropogenic column clean up, KCEL SOP #718 and #783. The PCB homologs are listed with their associated LLOQs for tissue samples in Table 8. LLOQs for PCB homologs are based on an initial sample weight of 15 (±0.05) g and a final volume of 0.5 ml. SW-846 method allows reporting of values below LLOQ if confirmed by mass spectrometry. These values would be considered estimated values and J qualified.

Table 8. PCB homolog targets and detection limit goals

Homolog group	LLOQ (µg/kg ww)
Monochlorobiphenyls	0.083
Dichlorobiphenyls	0.083
Trichlorobiphenyls	0.083
Tetrachlorobiphenyls	0.167
Pentachlorobiphenyls	0.167
Hexachlorobiphenyls	0.167
Heptachlorobiphenyls	0.250
Octachlorobiphenyls	0.250
Nonachlorobiphenyls	0.417
Decachlorobiphenyl	0.417

LLOQ = Lower Limit of Quantitation.

4.4 PBDE Congeners

PBDE congener analysis will follow KCEL SOP #781. The method relies on the quantitation of congeners using gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI). Fourteen most commonly detected congeners are quantified using this method. Sample preparation is described in SOP# 705 for soils, tissues, and sediments. The preparation method is a soxhlet technique following EPA method SW-846-3540C using methylene chloride as the extraction solvent. The PBDEs, pesticides and lipids will all be extracted together. The extract will be split 50-50, half for lipids determination and half for CLPEST/PBDE analyses. The CLPEST/PBDE half will have GPC, TBA, Alumina, and Acid cleanups performed per KCEL SOP #718, 721, 719, and 720 respectively. After cleanups the extract will be vialed at 0.5 mL for PBDE and 0.5 mL for pesticides. The PBDEs are listed with their associated detection limit goals for tissue samples in Table 9. LLOQs for PBDEs are based on an initial sample weight of 20 (±0.05) g and a final volume of 2.0 ml. SW-846 method allows reporting of values below LLOQ if confirmed by mass spectrometry. These values would be considered estimated values and J qualified.

Table 9. PBDE Congener Targets and Detection Limit Goals

BDE congener	Congener #	LLOQ (µg/kg ww)
2,2',4-TriBDE	17	0.04
2,4,4'-TriBDE	28/33	0.04
2,2',4,4'-TetraBDE	47	0.36
2,3',4,4'-TetraBDE	66	0.058
2,3',4',6-TetraBDE	71	0.04
2,2'3,4,4'-PentaBDE	85	0.04
2,2'4,4'5-PentaBDE	99	0.68
2,2',4,4'6-PentaBDE	100	0.112
2,2',3,4,4',5' HexaBDE	138	0.04
2,2',4,4',5,5'-HexaBDE	153	0.04
2,2',4,4',5',6-HexaBDE	154	0.044
2,2',3,4,4',5',6-HeptaBDE	183	0.04
2,3,3',4,4',5,6-HeptaBDE	190	0.04
2,2',3,3',4,4',5,5',6,6'-DecaBDE	209	0.5

LLOQ = Lower Limit of Quantitation.

4.5 Chlorinated Pesticides

Chlorinated pesticides analysis will follow KCEL SOP #733. The method relies on the quantitation of pesticides using gas chromatography/Electron Capture Detector (GC/ECD) by EPA Methods SW-846-8081B. Sample preparation is described SOP# 705 for soils, tissue, and sediments. The preparation method is a 20 gram to 2 ml fv soxhlet technique following EPA method SW846-3540C using methylene chloride as the extraction solvent. The PBDEs, pesticides and lipids will all be extracted together. The extract will be split 50–50, half for lipids determination and half for CLPEST/PBDE analyses. The CLPEST/PBDE half will have GPC, TBA, Alumina, and Acid cleanups performed per KCEL SOP #718, 721, 719, and 720 respectively. After cleanups the extract will be vialed at 0.5 mL for PBDE and 0.5 mL for pesticides. The targeted pesticides and associated detection limit goals are presented in Table 10.

Table 10. Chlorinated Pesticides Targeted and Detection Limit Goals.

Analyte	LLOQ (µg/kg ww)
Alpha-BHC	0.50
Beta-BHC	0.50
Delta-BHC	0.50
Gamma-BHC (Lindane)	0.50
4,4'-DDE	0.50
4,4'-DDD	0.50
4,4'-DDT	0.50
alpha-Chlordane	0.50
Trans-Chlordane	0.50
Aldrin	0.50
Heptachlor	0.50
Heptachlor Epoxide	0.50
Endosulfan I	0.50

LLOQ = Lower Limit of Quantitation.

4.6 Conventional Parameters

Total solids will be analyzed according to SM2540-G. The total solids analysis will follow KCEL SOP # 307. The MDL is 0.005 % and the RDL is 0.010 % for tissue samples. Lipid analysis will be conducted following KCEL draft SOP # 740. Samples are extracted by the same method as the PBDEs. The lipid analysis MDL and RDL are 0.05 and 0.1 %, respectively.

4.7 Laboratory Quality Control

This section presents laboratory quality assurance/quality control (QA/QC) measures that will be employed to ensure data are of sufficient quality to meet the project DQOs.
An analytical batch is defined as a maximum of 20 analytical samples plus QC samples. QC samples can include method blanks, spiked blanks, matrix spikes, matrix spike duplicates, lab duplicate, lab triplicates, lab control samples, lab control sample duplicates, SRMs and SRMDs (when available). For organic samples, surrogates are also added to every sample. QC samples will be analyzed at the frequency of one per QC batch, defined as up to 20 samples analyzed together. Standard Reference Materials (SRMs) will be used whenever available. Some analyses have empirically derived laboratory limits for various QC samples; those in place at the time of sample analysis will be followed. These will be included in the laboratory QC reports.

QC results that exceed the acceptance limits will be evaluated to determine appropriate corrective actions. Samples will typically be reanalyzed if the unacceptable QC results indicate a systematic problem with the overall analysis. Unacceptable QC results caused by a particular sample or matrix will not require reanalysis unless an allowed method

modification would improve the results. The project manager will be consulted prior to re-analysis. The laboratory QC sample requirements for each analysis are summarized in Table 11. The recommended QC limits for tissue analysis are in Table 12.

Table 11. Minimum quality control samples by analysis

Parameter	Blank[1]	Duplicate[2]	Matrix Spike	LCS and/ or SRM[3]	Surrogates	Spiked Blank
Metals	1 Per Batch	1 Per Batch	1 Per Batch	Yes	No	1 Per Batch
Mercury	1 Per Batch	1 Per Batch	1 Per Batch (MS/MSD)	Yes	No	1 Per Batch
PCB Homologs	1 Per Batch	N/A	1 Per Batch[4] (MS/MSD)	No	Yes[5]	1 Per Batch
PBDEs	1 Per Batch	N/A	1 Per Batch[4] (MS/MSD)	Yes	Yes	1 Per Batch
Chlorinated Pesticides	1 Per Batch	No	1 Per Batch[4] (MS/MSD)	Yes	Yes	1 Per Batch
Total Solids	1 Per Batch	1 Per Batch	No	No	No	No
Lipids	1 Per Batch	2 Per Batch of 20	No	No	No	No

N/A = not applicable

[1] Batch - A group of samples analyzed together for QC purposes containing a maximum of 20 samples.

[2] Duplicate - Triplicate analysis for all conventional parameters, duplicate analysis for metals and mercury.

[3] SRM - Standard reference material (must be certified by NIST or NRCC). LCS - Lab Control Sample.

[4] MS/MSD analyzed if sample volume allows, otherwise a spiked blank duplicate will be analyzed.

[5] Decachlorobiphenyl is a target analyte for PCB homologs

Table 12. Quality control limits for tissue samples.

Parameter	Blank[1]	Replicate[2]	Matrix Spike[3]	SRM[4]	Surrogates	Spiked Blank
Metals	< ½ LLOQ	≤ 20%	75 - 125%	varies	N/A	85 to 115%
Mercury	< ½ LLOQ	≤ 20%	75 - 125%	80 to 120%	N/A	85 to 115%
PCB Homologs	< ½ LLOQ	≤ 35%	Lab performance limits	N/A	Lab performance limits	Lab performance limits
PBDEs	< ½ LLOQ	≤ 35%	Lab performance limits	50 to 150%	Lab performance limits	50 to 150%
Chlorinated Pesticides	< ½ LLOQ	≤ 35%	50 - 150%	50 to 150%	Lab performance limits	50 to 150%

Parameter	Blank[1]	Replicate[2]	Matrix Spike[3]	SRM[4]	Surrogates	Spiked Blank
Lipids	<MDL	≤ 20%	N/A	N/A	N/A	N/A
Total Solids	< MDL	≤ 20%	N/A	N/A	N/A	N/A

NOTES:

[1]For SW-846 methods, the comparison for blanks is ½ the LLOQ value. For non-SW-846 methods, the concentrations in blanks shall be below the MDL.

[2]Relative percent difference (RPD) for duplicate analysis and percent relative standard deviation (%RSD) for triplicate analysis; triplicate analyses applies to total solids and lipids.

[3]Percent recovery for matrix spike, standard reference material, and surrogates.

[4]If SRM or LCS is available.

Empirically derived performance-based control limits may be updated once per calendar year and the limits in effect at the time of analysis will be used as QC limits for all ongoing precision and accuracy QC samples and surrogates.

N/A – not applicable

5.0 DATA ASSESSMENT, REPORTING AND RECORD KEEPING

This section presents how data related for crab tissue monitoring events will be reported and stored.

5.1 Data Assessment

Data assessment is critical for evaluating how well analytical data meet project DQOs. Data assessment is performed, at some level, during several steps in the process of sample analysis. Data assessment will also be performed by the KCEL QA Officer or Lab Project Manager for this program by reviewing complete data packages supplied by the KCEL. Data assessment will include a written narrative describing if the analytical data met the QA/QC requirements and a summary of laboratory data qualifiers with explanation of why data were qualified. This data assessment will be maintained along with the analytical data as part of the project records.

5.2 Reporting

Data will be reported in a technical memorandum or report that will include a presentation and interpretation of the crab tissue data results. The narrative will include summary data tables, descriptions of how the data were generated, including sampling and analysis descriptions. Data packages including the QA/QC data will be included as appendices.

5.3 Record Keeping

All hard-copy field sampling records, custody documents, electronic raw lab data, and laboratory summaries and narratives will be archived according to KCEL policy for a minimum of 10 years from the date samples were collected. A copy of the technical memorandum will be maintained in the DNRP Library.

6.0 REFERENCES

Carey, A.J., L.A. Niewolny, J.A. Lanksbury and J.E. West. 2014. Toxic Contaminants in Dungeness crab (*Metacarcinus magister*) and Spot Prawn (*Pandalus platyceros*) from Puget Sound, Washington, USA**.** Marine Resources Division, Washington Department of Fish and Wildlife. Olympia, WA

EPA 2000. Guidance for assessing chemical contaminant data for use in fish advisories. EPA No. 823-B-00-007. U.S. Environmental Protection Agency, Office of Water. Washington, D.C.

King County. 2014. 2014 Elliott Bay and Puget Sound Crab Tissue Monitoring Sampling and Analysis Plan. Prepared by Richard Jack and Debra Williston, Science and Technical Support Section, King County Water and Land Resources Division. Seattle, Washington.

King County. 2016a. Tissue Monitoring Program Work Plan. Prepared by the Toxics and Contaminant Assessment Unit, Water and Land Resources Division, King County Department of Natural Resources and Parks. Seattle, Washington.

King County. 2016b. Elliott Bay and Central Puget Sound Crab Data Report: 2014 Sampling. Prepared by Rory O'Rourke and Debra Williston, Water and Land Resources Division. Seattle, Washington. December 2016.

Kruse, G.H., D. Hicks, and M.C. Murphy. 1994. Handling increases mortality of softshell Dungeness crabs returned to the sea. Alaska Fishery Research Bulletin 1(1): 1-9.

Lippert, G., Hoffmann, S., and Clarke, P. 2002. Dungeness crab shell condition field manual. Washington Department of Fish and Wildlife. Olympia, Washington.

PSEP. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound. Prepared for US EPA and Puget Sound Water Quality Action Team by King County Water Pollution Control Division Environmental Laboratory (METRO Environmental Laboratory), under contract with the Puget Sound Water Quality Authority (PSWQA).

Velasquez, D. and D. Rothaus. Personal communication. 2018. Conference call between Rory O'Rourke, Jenée Colton, and Debra Williston, King County, with Don Velasquez and Don Rothaus of Washington Department of Fish and Wildlife, Olympia, WA, on 6 February 2018.

Washington Department of Fish and Wildlife (WDFW) website for soft-shell crab identification and handling. Accessed 13 April 2018. https://wdfw.wa.gov/fishing/shellfish/crab/softshell.html.

Appendix A

Field and Processing Sheets

Field Record for Elliott Bay/Puget Sound Crab Tissue Monitoring Event

Date of Collection: _____
Approximate Time: _____
Location: _____
Equipment: _____
Pot Number: _____
Pot Coordinates: _____
Approximate Water Depth: _____

Species[1]	# Taken	Returned		Observations[2]
		Number	Approximate size ranges	

[1] To lowest taxonomic level possible.
[2] Includes general disposition of species

Notes:_____
_____
_____
_____

Field Personnel: _____

Sample Processing for Elliott Bay/Puget Sound Crab Tissue Monitoring Event

Date of Collection: _____

Locator: _____

Species: _____

Pot Number: _____

Individual # (sequential)	Total Carapace Length (mm)	Sex	Whole Body Mass (g)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
	--	--	

Notes:_____
_____
_____
_____

Lab Personnel: _____

Sample Compositing Sheet for Elliott Bay/Puget Sound Crab Tissue Monitoring Event

Date of Collection(s): _____

Locator: _____

Species: _____

Tissue Type Muscle / Hepatopancreas

Sample ID: _____

Crab Pot #	Individual # (sequential)[1]	Total Carapace Length (mm)	Sex	Mass (g) Aliquot
Totals		--	--	

[1] From sample processing sheet.

Notes:_____
_____
_____
_____

Lab Personnel: _____

Appendix B

Scientific Collection Permit

WASHINGTON STATE
SCIENTIFIC COLLECTION PERMIT
Washington Department of Fish and Wildlife

*Please see **SCIENTIFIC/EDUCATION COLLECTION PERMIT (SCP) INSTRUCTIONS***
An Annual Report must be received before a renewal permit can be issued.
Applications and annual reports must be submitted via e-mail to scp@dfw.wa.gov.

If you have questions, please contact:
WDFW Licensing Division
Attn: SCP
PO Box 43154
Olympia, WA 98504
***Phone:** (360) 902-2464, Option 4*
***E-mail:** scp@dfw.wa.gov*

Permit Number (WDFW Use Only): **EASH-LOUCKS 18-095**

WHO:				
	1. APPLICANT INFORMATION			
	Name: Wendy Eash-Loucks	Agency: King County		
	Phone Number: 206-477-4683	Mailing Address: 201 S Jackson St, M.S. 600		
	E-mail: wendy.eash-loucks@kingcounty.gov	City: Seattle	State: WA	
		Zip Code: 98104		
	2. SUB-PERMIT HOLDERS			
	NAME/PHONE NUMBER:	**NAME/PHONE NUMBER:**		
	Christopher Barnes (206) 477-7143	Jean Power (206) 477-7149		
	Benjamin Budka (206) 477-7142	David Robinson (206) 477-7150		
	Jim Devereaux (206) 477-7144	Kimberle Stark (206) 477-4829		
	Jeff Droker (206) 477-7145	Lyndsey Swanson (206) 477-7121		
	Houston Flores (206) 477-5192	Debra Williston (206) 477-4850		
	Stephanie Hess (206) 477-7146	Rory O'Rourke (206) 477-7769		
	Dan Hutchens (206) 477-7720	Jenee Colton (206) 477-4075		
	Stephanie Jaeger (206) 477-5293	Deb Lester (206) 477-4752		
	Bob Kruger (206) 477-7147	Chris Gregersen (206) 477-4699		
	Marc Patten (206) 477-7148	Daniel Lantz (206) 477-4746		
	3. THIS APPLICATION IS:			
	☐ New	☒ Renewal of last year's Permit # Eash-Loucks 17-096a		
	☐ Amendment to Permit #			
	4. PURPOSE OF COLLECTION OR HANDLING			
	☐ Instruction/Education Display			
	☒ Research/Scientific Investigation (includes ELECTROFISHING)			
	☐ Salvage (deceased animals only)			
	☐ Fish Rescue/Relocation			
	5. RESEARCH OBJECTIVES			
	☐ Aging	☐ Behavior	☐ Physiology	☐ Artificial Propagation
	☒ Census	☐ Presence/Absence	☒ Population Distribution	☐ Life History
	☐ Pathology	☐ Genetic	☐ Stream Typing	
	☒ Other: Laboratory analysis of chemical contaminant concentrations (crab tissue)			

WHEN:	6. PERMIT TIMELINE*	
	Project Start Date: 4/22/2018	Project End Date:4/22/2019
	Permit Expiration Date (WDFW Use Only): **4/21/2019**	*SCPs are valid for a maximum of 12 months.

WHAT:	7. TYPE OF ANIMALS TO BE COLLECTED OR HANDLED				
	☐ Wildlife*	☐ Fish	☒ Aquatic Invertebrates – specify:	☒ Marine	☐ Freshwater

8. SPECIFIC TYPE(S)

Wildlife	**Fish**	
☐ Non-Raptor Birds	☐ Marine Fishes	☐ State and/or Federal Threatened or Endangered Species
☐ Raptors	☐ Freshwater Fishes	
☐ Mammals		
☐ Bats		
☐ Terrestrial Invertebrates		
☐ Reptiles/Amphibians		

9. COLLECTION INFORMATION

Species Requested – Both Common & Scientific names are required.	Specific Location & County Stream Section for Fish **County must be included**	Max # of Lethal Take or Live Permanent Removal	Max # of Non-lethal Take or Salvage
Zooplankton	Puget Sound Central Basin, **King** County	Up to 50 million	
Dungeness crab (*Metacarcinus magister*), male only, hard shell only	Near the following locations: Seacrest Park and Duwamish Head in West Seattle, Elliott Bay pier at Terminal 86 in Magnolia, Shilshole Bay Marina, Vashon Island at Quartermaster Harbor and Tramp Harbor, and Redondo fishing pier, all **King County**	65	0
Red rock crab (*Cancer productus*), both male and female, hard shell only	Near the following locations: Seacrest Park and Duwamish Head in West Seattle, Elliott Bay pier at Terminal 86 in Magnolia, Shilshole Bay Marina, Vashon Island at Quartermaster Harbor and Tramp Harbor, and Redondo fishing pier, all **King County**	65	0

HOW:	10. METHODS OF COLLECTION	
	☐ Firearms are being used for this collection.	
	Lethal Methods: Bongo Net/Oblique Tows (paired 60-cm nets, 335-µm mesh)	**Species:** Zooplankton
	Vertical Plankton Tows (60-cm ring net, 200-µm mesh)	Zooplankton
	Crab Pots	Dungeness crab (*Metacarcinus magister*)
	Crab Pots	Red rock crab (*Cancer productus*)
	Non-lethal Methods: n/a	**Species:** n/a

	Salvage Methods: n/a	**Species:** n/a
	Body-gripping traps: ☐ Padded Foot-hold ☐ Non-strangling type Foot Snare	**Species:** n/a
	Electrofishing: ☐ Backpack ☐ Boat	**Species:** n/a
	11. MARKING	
	☐ Band ☐ Mark ☐ Other	
	☐ Fit with radio/acoustic telemetry transmitters	
	12. DISPOSITION OF SPECIMENS	
	☐ Display Permanent – Dead	☐ Display Temporary - Dead
	☐ Display Permanent – Live	☐ Display Temporary – Live
	☐ Tissue sampling	
	☒ Laboratory use **(crab)**	
	☐ Live Housing Research of Laboratory use – Permanent	
	☐ Live Housing Research of Laboratory use – Temporary	
	☐ Immediate Release at Capture Site	
	☐ Relocated to Wild (additional permits may be required; wildlife may not be captured and relocated without a permit)	
	☐ Carcass disposal at site	☐ Display Temporary – Dead
	☐ Euthanize	
	☒ Other: **Preserved and identified by Julie Keister at the UW (zooplankton) and temporarily stored in reference/voucher collections**	

GENERAL PERMIT CONDITIONS:

1. A Scientific Collection Permit is non-transferable.
2. A copy of this permit must be in the possession of any person exercising the privileges authorized by this permit.
3. The Permit Holder is responsible for ensuring that all Sub-Permit Holders are qualified and experienced to conduct the specified activities, including collection by firearms and comply with all conditions of this permit. Only those Sub-Permit Holder(s) listed on the permit are authorized to engage in permitted activities.
4. Please note that compliance with Scientific Collection Permit requirements and permit conditions does not ensure compliance with federal, local, or other state laws. Collection of state or federal endangered or threatened species, state sensitive species, or state or federal candidate species is prohibited unless specifically authorized in this permit. Collection of game birds or game animals is prohibited unless specifically authorized in this permit. Collection of migratory birds, marine mammals, and any species listed under the federal Endangered Species Act may require a federal permit before collecting.
   For any collection/research activity of marine mammals and/or federally-protected anadromous and marine fish species, etc., contact NOAA-National Marine Fisheries Service at http://www.nmfs.noaa.gov/endangered.htm or 503-230-5400. For any collection/research activity of migratory birds, resident fish species (Bull Trout) and/or federally-protected wildlife, contact U.S. Fish and Wildlife Service at http://endangered.fws.gov or 360-753-9440.
5. This permit does not authorize collection from **non-WDFW** protected lands or waters (may include but not exclusive to: parks, reserves, refuges, natural areas, conservation areas, tribal lands, monuments, etc.). This permit does not authorize trespassing on private or restricted public lands. Additional permits issued by other state and local agencies, tribal governments, or landowners/managers may be required.
6. No collection shall occur in WDFW Marine Preserves or Conservation Areas (see http://wdfw.wa.gov/fishing/mpa), or Wildlife Areas unless permission is obtained from the Area manager. Contact the appropriate WDFW Regional Office for information. Regional office information is listed at http://wdfw.wa.gov/about/regions.
7. Specimens acquired under this permit remain the property of the state and will not be offered for sale or sold or used for commercial purposes or human consumption. Exchange or transfer of specimens, unless otherwise specified in this permit, requires prior written approval from the Director of WDFW.
8. Employees of WDFW have the right to inspect the collection activities authorized by this permit.

9. Vessels engaged in collection activities shall display a sign "RESEARCH," readable at 100 feet to unaided vision.

10. Permit Holders using unattended equipment must have attached to that equipment, a tag clearly marked with the permit number and name and current address of the Permit Holder. The address used may be that of the organization the Permit Holder represents, e.g., university, company, or corporation.

11. Permit holders may only use FDA approved fish anesthesia.
    a) **MS-222** may not be used at times and in places where fish may be subject to "catch and keep" fisheries within 21 days;
    b) **Clove oil** may not be used at all;
    c) **AQUI-S®** may be used as an alternative to MS-222.
        i. To use AQUI-S® 20E as an immediate release sedative in freshwater fish for field-based activities, permit holder must sign up to participate in USFWS-AADAP INAD 11-741 and must comply with the requirements as set forth in the INAD Study Protocol for AQUI-S® 20E (for more information about aquatic animal drugs, AQUI-S® 20E, or to apply to participate in USFWS-AADAP INAD 11-741 go to www.fws.gov/fisheries/aadap or contact the USFWS-AADAP INAD Administrator Bonnie Johnson at Bonnie Johnston@fws.gov or 406-994-9905).
    d) Carbon dioxide can be used as a fish anesthetic as per FDA rules and requires no withdrawal time;
    e) As alternative to chemicals, electro-anesthesia can be used as a fish anesthetic and requires no withdrawal time.

12. Unless otherwise specified in this permit, release of specimens is allowed only at the exact capture site immediately after capture. Release of fish and marine and freshwater invertebrates at any other site or time requires a transport, release, or planting permit. Relocating wildlife and releasing wildlife other than at the location of capture requires a special permit. The conditions of this permit may specify that no release of certain specimens is allowed. Contact WDFW Fish Program (360-902-2700) or Wildlife Program (360-902-2515) for further information.

13. Temporary holding of wildlife is permitted for identification only. Individuals must be released at site of capture, unless they exhibit evidence of disease.

14. At least 72 hours prior to initiating collection activities, the permit holder shall notify the Regional Wildlife Program Manager with the specific collection locations, dates, and times. (See the attached list of WDFW contacts and phone numbers.)

15. Wildlife Salvage — Notify the WDFW immediately if any State or Federally listed Threatened or Endangered species are encountered or salvaged and any salvaged State or Federal Threatened or Endangered Species must go to a major research collection such as WSU Conner Museum, University of Puget Sound Slater Museum of natural History, or UW Seattle Burke Museum, or as directed by the WDFW.

Reporting Requirements:

Permit renewal is contingent upon submission of a complete Annual Report. Reports must be submitted to WDFW upon completion of the display, education, or research project or the expiration date of the permit, whichever comes first, and must be received no later than 60 days after the expiration of the permit. All reports submitted to WDFW shall include Permit Holder's name and permit number and all required information on the Annual Report Form.

For **anadromous fish and freshwater collections,** the report shall include the 1) Date of collection; 2) Species name (for invertebrates, to the lowest taxonomic level possible); 3) Numbers of each species encountered and/or retained; 4) Location of each sample site, including county, water body, and latitude/longitude or GPS coordinates; 5) Disposition of specimens. This information is to be recorded at each capture site and includes ALL species encountered (or impacted by the collection activity) even if not retained or meant for the study

For **marine collections,** the report shall include the 1) Date of collection; 2) Species name (to the lowest taxonomic level possible); 3) Numbers of each species encountered and/or retained; 4) Location of each sample site, including county, water body, and latitude/longitude or GPS coordinates; 5) Disposition of specimens. This information is to be recorded at each capture site and includes ALL classified and unclassified species encountered (or impacted by the collection activity) even if not retained or meant for the study.

IN ADDITION for:

i. Rock scallops *(Crassodoma gigantea)* include: specific location, mortality of any rock scallop during collection, exact position and depth of specimens collected, and shell length measured from edge to edge at the widest part of the shell.

ii. Octopus *(Enteroctopus dofleini)* include: specific location, individual weight, depth, and sex of octopus taken.

For **wildlife collections,** the annual report shall include all categories on the Annual Report Form including the 1) Date of collection; 2) Species name (common and scientific) with numbers collected, numbers released, and disposition of individuals; 3) Location of collection including GPS coordinates, number of accidental mortalities.

SPECIAL CONDITIONS:

To prevent the spreading of aquatic invasive species, permit holder shall follow the procedures in the attachment, WDFW Protocols for Field Work Version 2 dated November 2012 (or the latest version of this document). For additional information on aquatic invasive species, please visit the WDFW website at http://wdfw.wa.gov/ais/.

Aquatic Invasive Species (AIS) Conditions:

Permit holder is required to humanely euthanize all collected aquatic invasive species (AIS) classified as "Prohibited aquatic animal species" under WAC 220-12-090 except as noted below for transport purposes. Collection of all Prohibited level 1 species[1] must be reported immediately to WDFW with photos of the species and specimens saved until provided to WDFW or directed to dispose. All other prohibited AIS must be euthanized before being removed from the immediate vicinity of the water body where collected and then disposed of in a public landfill system or chemically preserved. Collection and disposal of all other prohibited AIS must be included in a report submitted to WDFW within 30 days using the online reporting form noted below.

Permit holder may transport live prohibited AIS outside the immediate vicinity of the water body where collected only under the following conditions:

1. Transport to nearest WDFW regional office or headquarters for purpose of identification; AND
2. Transported in a secure container to prevent release of either the AIS or any associated water, plant, sediment, animal, or other materials; OR
3. Transported as authorized by a separate WDFW AIS Permit secured prior to collection.

Contact information:
WDFW Headquarters: 360-902-2700 and request Aquatic Invasive Species Unit Staff
Online reporting form: www.wdfw.wa.gov/ais/reporting
Toll-free: 888-933-9247

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[1] Includes: Zebra mussels (*Dreissena polymorpha*), quagga mussels (*Dreissena rostriformis bugensis*), European green crab (*Carcinus maenas*), and all members of the genus Eriocheir (including Chinese mitten crabs), all members of the walking catfish family (Clariidae), all members of the snakehead family (Channidae), silver carp (*Hypophthalmichthys molitrix*), largescale silver carp (*Hypophthalmichthys harmandi*), black carp (*Mylopharyngodon piceus*), and bighead carp (*Hypophthalmichthys nobilis*).

General Limitations for Marine Waters

1. Any sampling or collection in the marine waters of San Juan County or Cypress Island (Skagit County) must also be authorized by the director of the Friday Harbor Laboratories (FHL). The director (Billie Swalla (bjswalla@uw.edu)) of FHL has the authority under RCW 28B.20.320 to restrict or deny collections in these areas.
2. No work or collection is authorized in WDFW marine preserves or conservation areas. (See http://wdfw.wa.gov/fishing/mpa).
3. No collecting on public beaches (i.e., state, city, county, etc.) without the approval of the park manager (if one exists).
4. Collecting within a reservation requires tribal government approval.

Specific Limitations for Marine Invertebrates

1. Unless immediately released at site of capture, no live marine invertebrates (except for octopuses) may be returned to the wild without a transfer permit because of disease concerns. Contact WDFW shellfish biologist Brady Blake (360.302-3030, ext. 301 or Brady.Blake@dfw.wa.gov) for more information.
    a. Octopuses may be returned if they are in good shape and show no signs of disease.
2. Any marine invertebrates collected from waters within Clallam, Grays Harbor, Jefferson and Pacific Counties, may not be transported to another location or facility without having first obtained a shellfish transfer permit. Contact WDFW shellfish biologist Brady Blake (360.302-3030, ext. 301 or Brady.Blake@dfw.wa.gov) for more information.
3. Permit holder is restricted from transferring any marine invertebrates from any Restricted Shellfish Area. https://wdfw.wa.gov/licensing/shellfish_import_transfer/.
4. Any sea stars collected and transported from the wild cannot be returned to the wild and must be properly disposed of in an upland site.
5. Collection of the sunflower seastar (*Pycnopodia helianthoides*) is prohibited unless specifically authorized.
6. Permit holder is requested to report to WDFW (Brady Blake, Brady.Blake@dfw.wa.gov or 360.302.3030 ext. 301) any observations of distressed, dissolving, or fragmenting sea stars during their collections.
7. No marine invertebrates may be imported into Washington State without a shellfish import permit because of disease concerns. Contact WDFW shellfish biologist Brady Blake (360.302-3030, ext. 301 or Brady.Blake@dfw.wa.gov) for more information.

8. No cloud sponges (*Aphrocallistes vastus*) may be collected.
9. No box crab (*Lopholithodes foraminatus*) may be collected.
10. Collection of Puget Sound king crab (*Lopholithodes mandtii or L. foraminatus*) is prohibited unless specifically authorized.
11. No Olympia oysters (*Ostrea conchaphila* or *O. lurida*) may be collected from public beaches because they are in a recovery plan in Washington.
12. No pinto (northern) abalone (*Haliotis kamtschatkana*) may be collected.
13. Collection of red sea urchins, *Strongylocentrotus franciscanus*, green sea urchins, *Strongylocentrotus droebaciensis* and purple sea urchins, *Strongylocentrotus purpuratus* shall be limited to no more than 100 animals per species and ultimately no more than 10% of the observable population may be removed from any one site. If multiple collection sites are required, they should be separated by at least 5 miles in distance. No more than 20 purple sea urchins may be collected per site.
14. Collection of sea cucumbers (*Parastichopus californicus*) shall be limited to no more than 100 animals.
15. To minimize impacts on localized populations, collect no more than 20 individuals from any one intertidal locality.
16. Permit holder shall avoid collecting octopuses from within Octopus Conservation Areas and Octopus Protection Areas.
17. Female octopuses that are tending eggs may not be collected. In addition, the total number of octopuses collected, along with each individual's exact position, depth, and weight shall be recorded. A report of octopus harvest locations, the number taken, depth, and individual weights from each location shall be sent to WDFW shellfish biologist Henry Carson (360.902.2846, Henry.Carson@dfw.wa.gov) within 10 days of the last collection and should also be submitted with the annual report.
18. For geoducks (*Panopea generosa*) that will be collected using commercial harvest methods, the permit holder shall provide advance notice of planned geoduck harvest (date, time, specific location, vessel descriptions, and names of responsible personnel that will be at the harvest site with contact information such as cell phone numbers) to WDFW Enforcement (Enforcement.Office@dfw.wa.gov or 360.902.2936), WDFW Fish Program Customer Service (Program.FishManagement@dfw.wa.gov or 360.902.2700), WDFW shellfish biologist Henry Carson (Henry.Carson@dfw.wa.gov or 360.902.2846), and the Washington Department of Natural Resources (Linda Farr, Linda.Farr@dnr.wa.gov or 360.902.1053).

By signing below, the permittee agrees to abide by the conditions set forth in the Scientific Collections Permit issued by the Washington Department of Fish and Wildlife. Permittee agrees to all of the conditions outlined in WAC 220-20-045 and RCW 77.32.240. I also certify that if **firearms** are being used for collection under this permit all persons who will use firearms are legally capable of possessing firearms (per WAC 220-20-045(3)(e)).

An Annual Report must be submitted before renewal of next year's permit is granted. Under **RCW 77.15.660**, a violation of the terms or conditions of the scientific permit or any WDFW rule applicable to the issuance or use of the permit is a gross misdemeanor if the violation involves big game or big-game parts. It is also a gross misdemeanor under **RCW 77.15.660** to purchase or sell big game or big-game parts that were taken or acquired with a scientific permit. Under
RCW 77.15.160, a violation involving anything other than big game or big-game parts is an infraction.

This permit is not valid until signed by the permit holder and the WDFW Representative.

Signature of Permit Holder — Date: 4/9/18

Signature of WDFW Representative — Date: 4/10/2018

THIS PERMIT MAY BE REVOKED OR MODIFIED AT THE DISCRETION OF THE DIRECTOR OR THE DIRECTOR'S DESIGNEE.