# Quality Assurance Project Plan
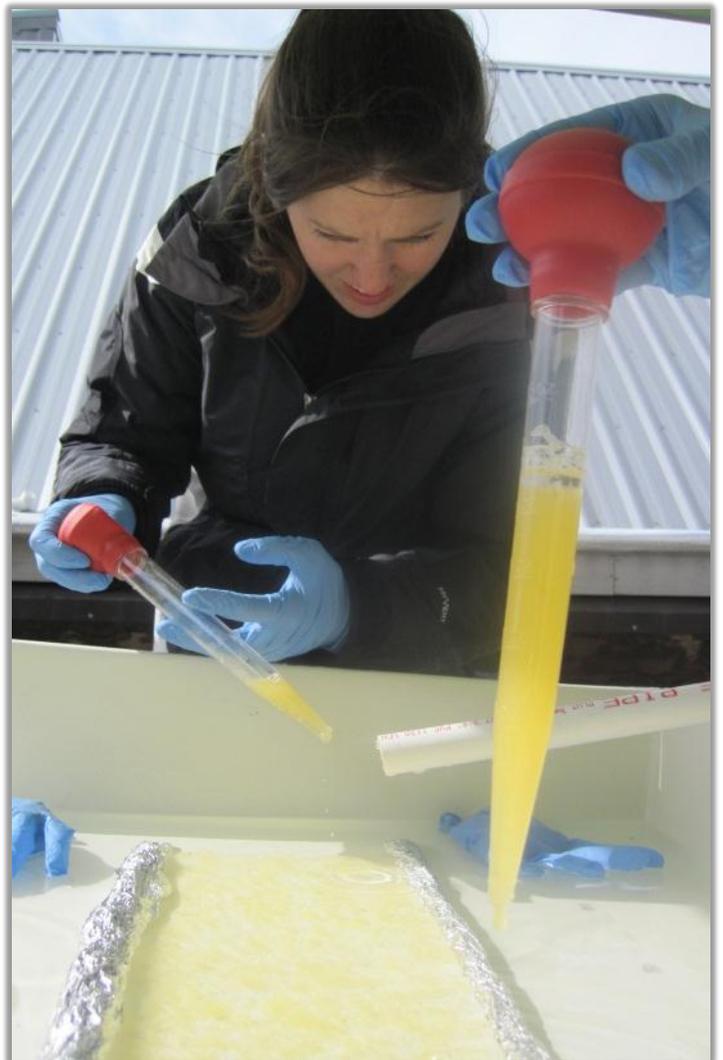
Effectiveness Monitoring for a Creosote-piling Removal Project: Embryos of Pacific Herring (*Clupea pallasi*) as Sentinels for the Presence of Polycyclic Aromatic Hydrocarbons (PAHs)



WDFW-DNR Interagency Agreement #13-120

February 8, 2013

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## 1.0    Quality Assurance Project Plan

# Effectiveness Monitoring for a Creosote-piling Removal Project: embryos of Pacific herring (*Clupea pallasi*) as sentinels for the presence of polycyclic aromatic hydrocarbons (PAHs)

## February 2012

**Approved by:**
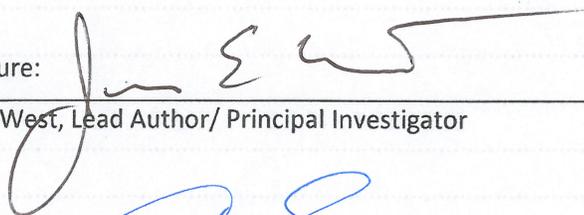
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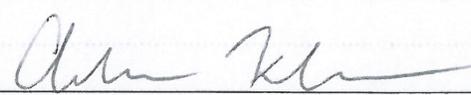

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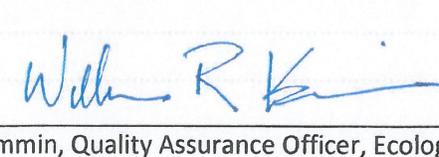

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# Contents

## List of Tables

## List of Figures

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## 2.0   Abstract

The Washington Department of Natural Resources (DNR) will remove approximately 346 derelict pilings from Quilcene Bay, Hood Canal, Washington from July 2013 to February 2014.  DNR has contracted with the Washington Department of Fish and Wildlife Puget Sound Ecosystem Monitoring Program's Toxics in Biota Unit (Toxics in Biota) to conduct the biological effectiveness monitoring portion of this effort.  The purpose of the herring egg study detailed here is to evaluate the effectiveness of removing these creosote-treated wooden pilings in reducing exposure of biota to chemical contamination from this source.  The project is designed to compare and document the exposure to chemicals, primarily polycyclic aromatic hydrocarbons (PAHs), on local biota (herring eggs) both before and after the Quilcene Bay creosote-treated pilings are removed.  This Quality Assurance Project Plan (QAPP) describes the objectives and operating procedures for this effectiveness monitoring study.

WDFW will collect ripe and running (spawn ready) Quilcene Bay herring in March 2013 and manually spawn gametes from captured adults onto controlled surfaces (nylon mesh) to create uniform units of spawned eggs from a common source.  The spawned eggs will be distributed among 25 anti-predator cages and then immediately deployed at various locations within the creosote-treated piling field.  Five replicates will be placed per sample distance and a control sample will be deployed at a site away from the piling field.  Developing embryos will be removed from the piling field after approximately ten days of incubation.  Upon retrieval eggs from each replicate sample will be placed into separate jars and analyzed for of a range of PAH contaminants.  This process will be repeated in the first spawning season after the creosote-treated piling removal, likely March of 2014.

Upon completion of the study, WDFW will produce a final report detailing the findings.  The final report will be posted to the internet and all data will be submitted for uploading into Ecology's Environmental Information Management database.

# 3.0    Background

This report details specific procedures and quality assurance guidelines proposed by the Washington Department of Fish and Wildlife *Toxics in Biota* staff to implement the following project: *Effectiveness Monitoring for a Creosote-piling Removal Project: embryos of Pacific herring (Clupea pallasi) as sentinels for the presence of polycyclic aromatic hydrocarbons (PAHs)*

As a member of the Puget Sound Ecosystem Monitoring Program (PSEMP), the Washington Department of Fish and Wildlife (WDFW) assesses status of and trends in the health of Puget Sound fishes and macro-invertebrates related to their exposure to toxic contaminants.  This *Toxics in Biota* effort is one component of PSEMP, a multi-agency effort designed to monitor the health of the Puget Sound ecosystem. PSEMP tracks a broad range of status indicators, including submerged aquatic vegetation, sediment health, fecal contamination in shellfish, water quality and several others.  WDFW's *Toxics in Biota* component of PSEMP (a) monitors the status and trends of chemical contamination in Puget Sound biota, (b) evaluates the effects of contamination on the health of these resources and (c) provides information to public health officials for assessing if Puget Sound seafood is safe to eat.

## 3.1 Study Area

This project is focused on a derelict creosote-treated piling (CTP) field in Quilcene Bay, Washington (Figure 1).  This CTP field exists within an area historically used by Pacific herring for spawning.  The CTP field has been targeted for removal and the study area comprises the piling field, as well as surrounding herring spawning habitat (eelgrass beds) within Quilcene Bay.

## 3.2 Logistical Problems

All studies of this type are subject to the normal rigors of conducting sampling in the field.  Difficult weather conditions can compromise sample quality or necessitate schedule changes.  SCUBA is required to set and retrieve the sampling units created for this study, which presents additional limitations in scheduling and logistics.  However, herring in this area spawn over a period of several weeks, so these potential logistical problems should be avoidable.

Accurate identification of piling positions will be challenging after pilings have been remove.  The degree of disturbance from the removal operation is unpredictable.  Benchmark stakes will be placed in strategic locations; stakes combined with GPS coordinates will be used to reconstruct piling positions after removal for the "after removal" samples.

## 3.3 History of the Study Area

Quilcene Bay and the surrounding waters of Hood Canal are considered some of the most pristine marine waters in the Puget Sound ecosystem.  The study area contains the remains of a trestle which was originally constructed to accommodate transferring materials from ship-to-shore along a shoreline lacking a deep-draft harbor.  This trestle may be a significant local source of PAH exposure to local organisms.

## 3.4 Contaminants of Concern

The primary contaminants of concern are polycyclic aromatic hydrocarbons (PAHs).  These contaminants are abundant in the creosote used to treat pilings and are highly toxic to marine life.  Developing herring embryos are particularly sensitive to PAH exposure, exhibiting a range of sublethal and lethal effects even at low doses. WDFW proposes to evaluate effects on developing herring embryos by comparing tissue residues of PAHs in embryos with published PAH effects thresholds such as those described by Carls et al. (1999). These authors reported a range of effects including yolk sac edema and premature hatching in herring embryos exposed to dissolved PAHs, resulting in tissue total PAH concentrations ranging from 22 to 108 ng/g wet weight.  Others have reported various developmental abnormalities and mortality associated with creosote (Vines et al., 2000), and PAHs from other sources such as oil spills (Incardona et al., 2009; Incardona et al., 2004), as well as increased toxicity of PAHs in embryos with exposure to natural sunlight (Barron et al., 2003; Hatlen et al., 2010; Incardona et al., 2012).

## 3.5 Results of Previous Studies

A study conducted by WDFW (PSEMP) have shown uptake of PAHs in herring embryos from several locations in Puget Sound (Wet et al. in prep).  This study documented uptake of PAHs in naturally spawned herring ranging from near zero (in Quilcene Bay) to concentrations exceeding 100 ng/g wet weight.  High mortality of embryos observed in locations with high PAH loads may have been related to PAH exposure.  From this previous work we suspect that naturally spawned Quilcene Bay herring embryos are not exposed to PAHs, except for areas where PAHs may be considered a point source, such as a CTP field in this case.

## 3.6 Regulatory Criteria

Although there are no criteria regulating the exposure of herring embryos to PAHs, the results of this study should allow some extrapolation of effects of proximity to creosote treated pilings on herring health, in developing recovery goals for Puget Sound.

# 4.0     Project Description

This project is designed to evaluate the effectiveness of removing creosote-treated wooden pilings in reducing potential exposure of biota to chemical contamination from this source in Puget Sound.   The Washington Department of Natural Resources (DNR) will remove approximately 346 derelict pilings from Quilcene Bay, in Hood Canal, Washington from July 2013 to February 2014.  DNR has contracted with DFW's PSEMP/Toxics in Biota Unit to conduct the biological effectiveness monitoring portion of this effort.

This effectiveness monitoring project is designed to demonstrate potential exposure of biota to chemicals from CTPs prior to CTP removal, and evaluate the degree to which that exposure has been reduced after CTP removal.  The basic intent of the effectiveness monitoring study is to (a) compare the exposure to chemicals (primarily PAHs from creosote) on local biota before CTPs are removed, and (b) document recovery of the organisms after removal.  The study will focus on PAH exposure to and effects on developing embryos of Pacific herring (*Clupea pallasi*) as its primary endpoint.  Pacific herring are a common and abundant small, schooling pelagic planktivore that spawn on nearshore vegetation in Puget Sound.  Developing embryos of fish such as herring and other nearshore spawners are particularly sensitive to exposure to low concentrations of dissolved PAHs (Carls et al., 1999; Heintz et al., 1999).  Herring embryos exhibit high mortality when spawned on or near creosote pilings (Vines et al., 2000).

The Quilcene Bay CTP field is ideal for this effectiveness monitoring tool because (a) Pacific herring are known to spawn on the eelgrass that grows interspersed with that piling field, (b) the CTP field covers a large area and so its potential for harm (and recovery) is large, and (c) the presence of other potentially confusing PAHs sources, such as stormwater runoff, is small for that location. To ensure control over where embryos occur in the piling field we propose to manipulate placement of embryos during the spawning season.  Caged herring embryos have been used successfully to monitor the exposure of herring embryos to PAHs from an oil spill and from background urban sources (Incardona et al., 2012).

## 4.1 Project Goal

The goals of this study are threefold: (a) measure the exposure of newly spawned herring embryos to PAHs from CTPs in Quilcene Bay, (b) compare the exposure to PAHs (as tissue residues) in embryos with distance from CTPs, and (c) evaluate the degree to which this exposure is reduced after CTPs have been removed.

## 4.2 Project Objectives

The objectives of this study are to (a) collect Quilcene Bay spawning herring in March 2013, (b) manually spawn gametes from captured adults onto controlled surfaces such as nylon mesh, (c) create uniform units of spawned eggs in anti-predator cages, (d) deploy up to 25 caged embryo sampling units (CESUs) at various distances from existing Quilcene CTPs, (e) remove developing embryos after approximately 10 days of incubation, and (f) process embryos for tissue residues of PAHs.  This process will be repeated in the first spawning season after CTP removal, likely March of 2014.

## 4.3 Information Needed and Sources

We will be generating new data on toxic contaminants in herring embryos, presented as wet weight concentration. Pre-existing PSEMP contaminant data on this species and life stage will be incorporated when pertinent, for context. Organic chemical contaminants including PAHs were measured by PSEMP/Toxics in Biota in naturally spawned herring embryos in Quilcene Bay in 2001. Results from that effort indicated Quilcene Bay herring embryos were among the least contaminated embryos from five stocks sampled in that study (WDFW unpublished data). This supports an assumption that Quilcene Bay is relatively uncontaminated overall, so PAH inputs at a small spatial scale (such as in a CTP field) should not be masked by ambient contaminant conditions. This study will provide similar, newer data on a smaller spatial scale, with embryos in close proximity to CTPs.

## 4.4 Target Population

The target population for this study is the Quilcene Bay stock of Pacific herring (*Clupea pallasi*).

## 4.5 Study Boundaries

The study will take place within the footprint of an area of high piling density (HPD) in the center of the piling field in Quilcene Bay, Hood Canal, Washington (Figure 1).

## 4.6 Tasks Required

Tasks involved in this study include:

- Collecting spawning adults
- Manually spawning herring onto controlled surfaces
- Deploying manually-spawned egg cages in the CTP field and reference areas
- Marking the location of recently extracted CTPs for future placement of monitoring cages
- Retrieving egg cages and transferring eggs to jars for chemical analysis
- Delivering samples to contract analytical lab
- QA/QC review
- Formatting data for relational database
- Analysis of data for PSEMP/DFW report
- Transfer of data to EIM
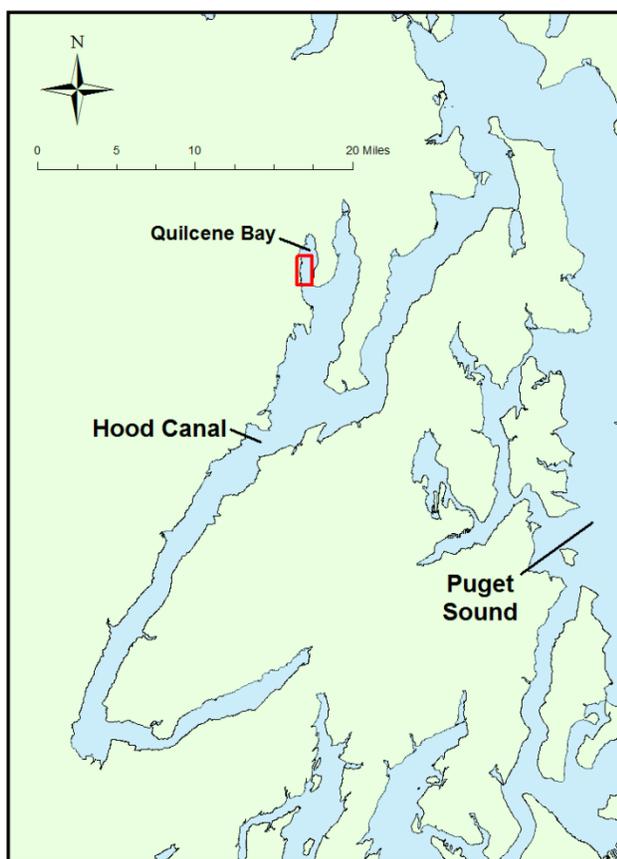


Figure 1. Location of Quilcene Bay, Washington

13

## 4.7 Practical Constraints

The most pertinent practical constraints here relate to (a) availability of Quilcene Bay stock spawning herring, (b) scheduling SCUBA divers to assist with field work, and (c) weather conditions that may impede field work.  The Quilcene Bay spawning stock is one of the most abundant stocks in the Puget Sound Basin, and they spawn over a three month period.  In addition, professional SCUBA divers are available from DFW during the months of herring spawning.  Thus these constraints will likely be small.

# 5.0    Organization and Schedule

## 5.1 Key Individuals and Their Responsibilities

Table 1. Organization of project staff and responsibilities.

| Name | Title | Phone # | Email | Responsibilities |
|---|---|---|---|---|
| James E. West | Senior Research Scientist | 360.902.2842 | james.west@dfw.wa.gov | Principal Investigator and lead author |
| Jennifer A. Lanksbury | Fish and Wildlife Biologist 3 | 360.902.2820 | jennifer.lanksbury@dfw.wa.gov | Co-investigator |
| Laurie A. Niewolny | Fish and Wildlife Biologist 2 | 360.902.2687 | laurie.niewolny@dfw.wa.gov | Project support, lab and field |
| Andrea Carey | Fish and Wildlife Biologist 2 | 360.902.2849 | andrea.carey@dfw.wa.gov | Project management, lab/field lead |
| Stefanie Orlaineta | Part-time temporary technician | 360.902.2657 | stefanie.orlaineta@dfw.wa.gov | Project support, lab and field |
| Tom Gries, | NEP QA Coordinator | 360.407.6327 | tgri461@ecy.wa.gov | reviews QAPP and draft report |
| William Kammin | Ecology QA Officer | 360.407.6964 | wkam461@ecy.wa.gov | approves QAPP |

## 5.2 Project Schedule

**Table 2. Proposed schedule for completing field and laboratory work**

| Field and laboratory work | Due date | Lead staff |
|---|---|---|
| Field work completed | April 15, 2013 (pre-CTP removal), April 15, 2014 (post CTP removal) | Jim West |
| Laboratory analyses completed | 30 May, 2014 | |
| Quarterly reports | | |
| Author lead | James West | |
| Schedule<br>QAPP approved – 15 February March 1?, 2013<br>Field Sample Summary Report -- 31 June, 2014<br>Complete lab analysis – 31 July, 2014<br>Final Report -- 30 Sep, 2014 | | |
| 1st quarterly report | Short progress report with invoice | |
| 2nd quarterly report | Short progress report with invoice | |
| 3rd quarterly report | Short progress report with invoice | |
| 4th quarterly report | Short progress report with invoice | |
| Final report | | |
| Author lead and support staff | James West, Jennifer Lanksbury, Laurie Niewolny, and Andrea Carey | |
| Schedule | | |
| Draft due to peer reviewers and NEP staff | July/August 2014 | |
| Final report due | 30 September, 2014 | |

## 5.3 Budget and Funding

This project is supported by an Interagency agreement with the WDNR.  WDNR is the primary contractor for the piling removal effort, as funded by Ecology as Lead Organization for Toxics and Nutrients Prevention, Reduction, and Control.   This overall effort is funded by EPA's National Estuary Program (NEP).  Match for this study is provided by WDFW in the form of staff time, vessel use, and laboratory supplies.

**Table 3. Proposed WDFW budget for 2013/14 herring egg study.**

| Item | Cost | Unit | Before Piling Removal | After Piling Removal | Total |
|---|---|---|---|---|---|
| DFW Biologist (II) | $5,343 | 4 months | $8,015 | $13,358 | $21,372 |
| DFW Technician | $4,471 | 1.5 months | $4,471 | $2,236 | $6,707 |
| Personnel Fees | $254 | | $95 | $159 | $254 |
| Sampling/Processing | $4,000 | 2 surveys | $2,250 | $2,250 | $4,500 |
| Travel | $1,000 | 2 surveys | $750 | $750 | $1,500 |
| Lab PAH analysis | $607 | 60 samples | $18,210 | $18,210 | $36,420 |
| Subtotal | | | $33,791 | $36,962 | $70,753 |
| WDFW indirect | 28.36% | | $9,583 | $10,482 | $20,065 |
| Pre-contingency total | | | $43,374 | $47,444 | $90,818 |
| Contingency | 10% | | $4,300 | $4,700 | $8,980 |
| **TOTALS** | | | **$47,674** | **$52,144** | **$99,818** |

# 6.0      Quality Objectives

## 6.1 Measurement Quality Objectives

The following Figure 2 (taken from Sloan et al., 2006, Table 8) lists the minimum QA criteria for PAHs analyzed in herring embryos for this study. PAHs are synonymous with Polycyclic Aromatic Compounds (PACs) in this table.

Table 8. Minimum analytical quality assurance criteria: Polycyclic aromatic compounds (PACs) and persistent organic pollutants (POPs) by gas chromatography/mass spectrometry (GC/MS).

| Quality assurance element | Minimum frequency | Acceptance criteria |
|---|---|---|
| Instrument calibration | Once every batch of samples or once every two batches in one continuous analytical sequence | Analyte concentrations are to be calculated using point-to-point calibration with at least four concentration levels of calibration standards. |
| Continuing calibration | At start and end of every analytical sequence and every 10 or fewer field samples | The RSD of the analyte responses relative to the internal standard is to be ≤ 15% for the repetitions. |
| Reference materials: Sediment: NIST SRM 1944, NIST SRM 1941b Mussel tissue: NIST SRM 1974b Blubber: NIST SRM 1945 Fish tissue: NIST SRM 1946, NIST SRM 1947 | One with every batch of 20 or fewer field samples | Concentrations of ≥ 70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values. These criteria do not apply to analytes with concentrations below their lower LOQ when the lower LOQ is within or greater than the 95% confidence interval, nor to those analytes known to have coeluting compounds. |
| Method blank | One with every batch of 20 or fewer field samples | No more than 5 analytes in a method blank are to exceed 2 × lower LOQ. Samples are not corrected for analytes found in the blank. |
| Sample replicates (i.e., duplicates or triplicates) | One with every 20 or fewer field samples | RSDs are to be ≤ 15% (equivalent to relative percent difference ≤ 30% for duplicates) for ≥ 90% of the analytes that have concentrations ≥ 1 ng/g. |
| Internal standards/surrogates | At least one internal standard/ surrogate is added to every sample | The recoveries are to be 60–130%. |
| Interlaboratory comparisons | At least one per year | In conjunction with the NIST or the IAEA. |

**Figure 2. Minimum analytical quality assurance criteria reproduced from Sloan et al. 2006.**

Measurement quality objectives for bias associated with measurement of % lipids are that each NIST SRM result should be within its control limits (Sloan et al, 2006):

- Upper control limit = [1.35 × (certified concentration + uncertainty value for 95% confidence)]
- Lower control limit = [ 0.65 × (certified concentration – uncertainty value for 95% confidence)]

The measurement quality objective for % solids is drying samples to a constant weight.

## 6.2 Precision

Precision is monitored and controlled within batches using laboratory replicates of field samples and across batches by analyzing Standard Reference Materials (SRM) of applicable matrix i.e., tissue.   For this study [NIST SRM 1974b](#) will be used for all organics[1].  Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be ≤ 15% for the repetitions.

## 6.3 Bias

Bias or accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values.  In addition for POPs, concentrations of ≥70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values.

## 6.4 Sensitivity

The Lower Limit of Quantitation (LOQ) for all PAHs in this study is "the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration.  When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ."  (Sloan et al. 2006). Typically LOQ values for PAHs that have been reported to PSEMP by this method are in the range of 0.2 to 0.8 ng/g wet weight.  In this study, the PAHs' LOQs are given as a range because tissue sample LOQs are affected by the field sample mass used.  The LOQ is the lowest concentration at which a PAH's sample result will be reported.

## 6.5 Comparability

The SOPs described in this document (Sloan et al., 2004; Sloan et al., 2006) are consistent with other concurrent and future sampling efforts that could be used as comparison for herring eggs.

## 6.6 Representativeness

The sampling design in this study is aimed at representing contaminant conditions as tissue residues in herring eggs across a gradient of potential PAH exposure.  To that end the design optimizes spatial coverage that represents conditions ranging from close to (sample cage is touching) to far away (approximately 1000 m) from the existing Quilcene CTP field.  Quilcene herring are known to spawn around or on these pilings in this field, and so the location of planned cage placement is congruent with normal spawning behavior.  In addition, spawning adults will be sampled during their peak spawning period for that stock, to maximize representativeness of gamete quality.

The degree to which results from this study will represent other stocks exposed to PAHs from CTP fields is unpredictable, because of variable CTP age (and PAH weathering), local conditions, and spawning

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[1] SRM 1974b is no longer available from NIST.  The NOAA lab has enough matrix on hand for this study, however, a suitable alternative may be substituted, at the chemist's discretion.

behavior of herring throughout Puget Sound.  However, we expect this study to yield some basic tenets regarding PAH exposure of a sensitive life stage in Puget Sound, relative to CTPs as a source of PAHs.

## 6.7 Completeness

This study will be considered complete if sufficient CESUs are retrieved after 10 days of incubation to represent a gradient of conditions from near-to-piling, to well away from pilings, with a minimum of three replicates per distance unit.   This condition must be repeated after CTPs are removed, with pre-removal locations reoccupied.

# 7.0    Sampling Process Design (Experimental Design)

## 7.1 Overall Study Design

The study is designed to address the question "what is the relationship between proximity to creosote pilings and the health of developing herring embryos?"  This question generates the null hypothesis; there is no relationship between tissue residue of PAHs in developing herring embryos with distance from creosote pilings.  In addition, we plan to repeat the sampling subsequent to piling removal, which addresses the question, "does the removal of creosote pilings reduce or eliminate exposure to PAHs of herring embryos developing nearby?"  The null hypothesis for the second sampling is "there is no

significant difference in PAH exposure to herring embryos developing at locations where creosote-treated pilings had recently been removed, and those developing at a distance from the previous piling field".  These questions will be address by manipulating the fine-scale placement of developing herring embryos deployed in small anti-predator cages relative to the existing Quilcene piling field.

## 7.2 Sampling location and frequency

All Caged Embryo Sampling Units (CESUs) for this study will be created on the same day, from eggs and milt mixed at the same time.  One sampling event will occur prior to the piling removal (the 2013 "before" sample) and one sampling event will occur subsequent to the piling removal (the 2014 "after" sample).  The actual dates of piling removal are uncertain, but are
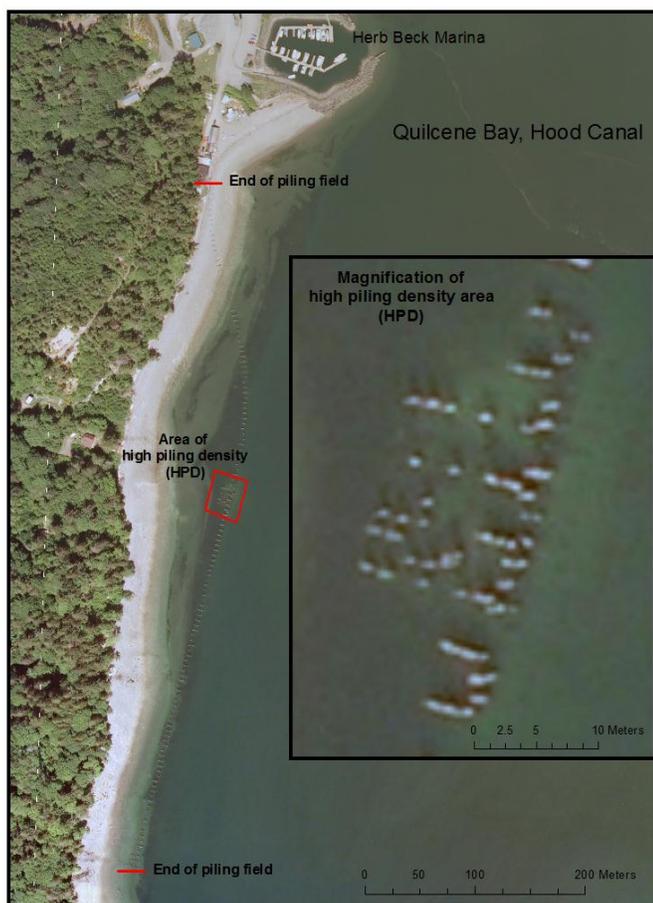


Figure 3.Creosote piling field, once a train trestle, with inset of high piling density (HPD) study focus area.

20

targeted for the summer of 2013. The after sampling will be conducted during the first normal herring spawning event following CTP removal (i.e. the winter of 2014). All CESUs will be deployed within the piling field or within the confines of Quilcene Bay (for the reference samples) where herring typically spawn.

The piling field, which is the remnant of a train trestle, comprises a line of approximately 350 pilings running parallel to the shoreline in a north-south direction for roughly 645 meters (Figure 3). Near the center of this north-south piling line is an area of high piling density (HPD) measuring roughly 246 m$^2$, which we will use as our sampling area.

Within the center of the HPD area in 2013 we will place replicate cages with herring eggs at four discrete distances from pilings; a) touching (i.e. attached to) pilings, b) 0.30 m, c) 1.0 m, and d) 2.0 m away from pilings (Table 4). These distances were selected based on conversations with Mark Carls (NOAA Fisheries, Auke Bay Lab) and Dr. Gary Cherr (University of California, Bodega Marine Laboratory). Current and past field work conducted by these researchers using embryos and polyethylene membrane devices suggest the 100 cm scale should be sufficient to capture a PAH gradient if it exists.

The above deployment scheme will be repeated in 2014 after CTP removal, with placement relative to locations where pilings had previously existed. At all sites the caged embryos will be suspended approximately 10 cm (4 inches) above the sediment, to mimic the depth at which herring eggs are typically laid.

Three reference or control sampling efforts will be conducted. Firstly we plan to elucidate the pattern of PAHs (as a fingerprint) from the Quilcene CTPs by taking three replicate samples of wood from pilings used in the study in 2013. This will be used to compare the fingerprint, or relative abundance of PAH analytes in embryos. Secondly we will sample naturally occurring herring embryos from a reference site in 2013 to describe the natural background concentration of PAHs in naturally spawned herring embryos incubating in natural habitat, far removed from any putative PAH sources. This control will be used to confirm previous observations and test the assumption that PAHs in background conditions are trivial. Thirdly we will test whether the manual spawning and caging of herring embryos exposes them to PAHs in both years. This control is used to ensure that the manual spawning, CESU creation, and deployment procedures do not contaminate embryos with PAHs. These last controls will be randomly selected from CESUs just prior to field deployment, and will also serve as an estimate of the initial PAH condition (if any) of embryos deployed in the CESUs.

**Table 4. Monitoring design: samples sizes by type of sample.**

| | | | |
|---|---|---|---|
| Touching piling/over piling footprint* | Caged eggs | 5 | 5 |
| 0.25 meter away from piling | Caged eggs | 5 | 5 |
| 0.5 meter away from piling | Caged eggs | 5 | 5 |
| 1 meter away from piling | Caged eggs | 5 | 5 |
| Reference site <1000 m from CTP field | Caged eggs | 3 | 3 |
| Reference site <1000 m from CTP field | Naturally laid eggs | 3 | |
| Creosote piling sample | Wood | 3 | |
| QC samples | NA | 4 | 4 |
| | Total | 33 | 27 |

*Prior to CTP removal five replicate cages will be touching (directly adjacent to) pilings. After CTP removal the five replicate cages will be placed directly over the footprint of previously standing pilings. To assist with accurate placement of the "over the footprint" cages, SCUBA divers will flag the divots left by recently removed pilings just after piling removal.

## 7.3 Map of study area

Figure 3 shows an overhead photo of the Quilcene bay shoreline with the derelict trestle-piling field. The inset is an enlarged view of the high piling density (HPD) area, roughly in the center of the overall piling field, where this study is focused.

## 7.4 Parameters to be determined

Parameters to be determined from embryos recovered from the CESUs include the tissue concentration of PAH contaminants (see Table 5, Section 9.1) both before and after placement at various distances from pilings within the HPD area of the piling field and from reference/control samples. We will also determine the concentration of PAH contaminants in naturally laid eggs at a reference site and the PAH signature of wood taken directly off the creosote pilings. Additional metrics from embryos include total extractable lipids and total percent solids.

At time of retrieval a small subset of embryos will be removed from each CESU and evaluated for mortality. At least 100 embryos will be examined under a dissecting scope and number of dead embryos counted.

## 7.5 Field measurements

Field measurements related to capturing adults for gametes include date, time, location (latitude/longitude of the net midpoint. The hand-held GPS units (Garmin, GPSmap 76C, and GPSmap 176) available to PSAMP staff report coordinates to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft)., and method of capture, water depth, temperature, species, sex, standard and fork length (in mm). Field measurements related to cage deployment and retrieval will include date, time, water temperature and salinity, sample location (distance from nearest piling, Table 4), site replicate number and water depth (corrected to Mean Lower Low Water (MLLW)). Individual pilings to which CESUs are attached will be identified with plastic tags attached above water with roofing nails. A detailed map of CESU placements with distances to all nearby pilings will be made using a tape measure above water.

## 7.6 Assumptions underlying design

We assume that manually spawned caged embryos will absorb dissolved PAHs, if present, in the same manner as naturally spawned embryos on natural habitat. Manually spawned herring embryos have long been used as model organisms for bioassay studies (Dinnel et al., 2011), and caged embryos have been used as PAH monitors for damage assessment related to oil spills (Incardona et al., 2011). In addition (Vines et al., 2000) recorded effects of creosote on herring embryos spawned on CTPs.

We assume maternal transfer of PAH chemicals to eggs will be low enough to be inconsequential for Quilcene Bay herring. This is supported by multiple lines of circumstantial evidence, including a) measurement of PAHs in ovaries of herring from the nearby Port Gamble stock, within which PAHs were only rarely detected (4 of 31 compounds), at sub part-per-billion concentration (wet weight, PSEMP unpublished data), b) trivial tissue residues of PAHs in herring prey species (krill, *Euphausia pacifica*) and phytoplankton from near Quilcene Bay, compared to other Puget Sound basins (West et al., 2011), c) Quilcene Bay herring embryos were among the least PAH-contaminated embryos from five stocks sampled in a 2001 field study (WDFW, unpublished data), and d) trivial concentration of PAHs in herring ovaries from adult stocks (in Central Puget Sound) exposed to PAHs (WDFW unpublished data). Unlike many bioaccumulative contaminants such as polychlorinated biphenyls, PAHs are typically effectively metabolized by vertebrates including fish, and so are typically not transferred from parent to egg.

## 7.7 Relation to objectives and site characteristics

Characteristics of the Quilcene piling field are ideal for this effectiveness monitoring study. Quilcene Bay, including subtidal areas around the piling field, is well documented as spawning habitat for Pacific herring. Their spawn timing is highly predictable, with median deposition occurring in mid-March (Figure 4), averaged over the past 10 years. There are few potential local sources of PAHs except for a small marina, located approximately 450 m from the proposed study site. Boat access to the bay is readily available and relatively close to the study area. The Point Whitney Marine lab, which will be used during the creation of the CESUs is located nearby.

A consistent number of CESUs will be sampled from each of the targeted sampling locations in the Quilcene CTP field and the reference site, both before and after CTP removal. In addition, Quilcene herring are known to spawn on and around this CTP field at approximately the same depth as the cages will be placed.



Figure 4. Estimated spawn timing by week for Quilcene Bay herring stock, 2003-2012

## 7.8 Characteristics of existing data

Organic chemical contaminants including PAHs were measured by PSEMP/Toxics in Biota in naturally spawned herring embryos in Quilcene Bay in 2001. Results from that effort indicated Quilcene Bay herring embryos were among the least contaminated embryos from five stocks sampled in that study

(West et al. in prep).  Total PAH concentration in three composite samples of Quilcene Bay embryos from that study ranged from 1.1 to 6 ng/g wet weight, (mean of 3.7 ng/g) compared  to concentrations ranging to 140 ng/g from other locations.  Published PAH effects thresholds, such as those described by Carls et al. (1999), describe a range of sublethal and lethal effects in herring embryos exposed to dissolved PAHs, resulting in tissue total PAH concentrations ranging from 22 to 108 ng/g wet weight.

# 8.0    Sampling Procedures – Field and Lab

## 8.1 Field Measurements and Field Sampling Standard Operating Procedures

The SOP outlined below is adapted from three field SOPs that describe collection of adults to obtain gametes for spawning (Quinnell et al. in prep), manually spawning herring onto a controlled surface (Nitex mesh – (Dinnel et al., 2011), and creating and deploying Caged Embryo Sampling Units (CESUs - (Incardona et al., 2011).

### 8.1.1 Collecting adult herring for spawning

During the 2013 herring spawning season, ripe pre-spawn Quilcene Bay herring will be captured via boat with a gill net following procedures outlined in (Quinnell et al. in prep).  Briefly, this involves deploying one or more monofilament gill nets approximately 100 ft. x 8 ft. x 1.75 inch mesh.  The net(s) will be deployed at dusk in an area where fish have been spawning and checked every 30 to 60 minutes, depending on the number of fish being captured.

Nets will be hauled on board and fish carefully removed from the mesh.  The fish will immediately be sexed and those in spawning condition retained.  A fish is accepted as "in spawning condition" when milt or eggs can be expressed from the vent with gentle pressure applied to the fish's abdomen.  Spawn-ready fish will be transferred to Ziploc bags with sexes separated, placed on ice, and transported to a nearby marine laboratory.  Dissection of gonads and manual spawning will be carried out within 8 hours of collection per Dinnel (2011).  Data collected will include collection date, time, capture method, location, number of females and male fish collected, and temperature of the water from which the fish were collected.

### 8.1.2 Manually spawning herring

We will follow the methodology described in Dinnel et al. (2006) and (Dinnel et al., 2011).  Briefly, this involves excising the ovaries and testes from spawn-ready fish, and mixing them on Nitex mesh in trays filled with seawater.  Eggs will be removed from ovaries of approximately 20 females with a spatula and distributed evenly over mesh in spawning trays filled with local seawater kept at Quilcene Bay ambient temperature (Figure 5). Because eggs are adhesive they can be easily spread onto the mesh in a mono-layer, to which they will adhere.  When trays of unfertilized



Figure 5. Egg fertilization and cage construction in the laboratory.

Clockwise from left: replicate Nitex mesh sheets with monolayers of eggs incubating in milt; insertion of Nitex sheet with fertilized eggs into cage; fully assembled cage, or CESU. Illustrations courtesy John Incardona

eggs have been created in this fashion, milt will be prepared.  Sections of testes from at least five males will be excised and macerated in ambient seawater in a pre-cleaned glass jar to produce a sperm mixture which will then be poured into the egg tray.  After addition of the sperm solution the egg tray will be gently swirled to ensure all eggs come into contact with sperm.  The tray will then be covered with foil and allowed to sit for 60 minutes.  After 60 minutes water in the trays will be decanted and replaced with ambient Quilcene Bay seawater.  The trays will be incubated at Quilcene Bay temperatures for 8 to 12 hours until the Nitex mesh is removed for placement into the cages.  During this incubation period a subsample of approximately 100 eggs will be removed from each spawn-tray, and assessed for fertilization rate.  Fertilization rate will be obtained by comparing the number of eggs that have undergone first cleavage (Hill and Johnston, 1997) with the total number of eggs.  A spawn batch will be considered successful if the fertilization rate is greater than 70%.  If the ratio falls below the above standard, the process will be repeated with fresh gametes.

### 8.1.3 Creating Caged Embryo Sampling Units (CESUs)

The pre-cut Nitex sheets with fertilized eggs will be placed into the cages with the eggs facing inward.  The lid of the cage will be secured with small zip ties in a position midway along the length of the lid, ensuring that there are no loose Nitex edges or zip tie ends protruding into the cage that could damage or scrape eggs off the mesh.  We will then attach a sample identification number to the lid of the cage.  The cage will then be placed into a large Ziploc bag and the bag filled with ambient Quilcene Bay seawater for transport to the site.

### 8.1.4 Deploying CESUs

Three CESUs will be attached using zip ties to a single length of precut rebar approximately 120 cm in length.  CESUs will be attached along the bar at zero, 30, and 100cm from the CTP.   A fourth CSEU will be attached to vertical stake at 200cm from the CTP.  The end of the bar with the zero-distance CESU will have its tip bent at a right angle to facilitate attachment to the piling.  Once attached the entire apparatus can be handled as a single unit.  All CESUs will be handled to avoid direct or prolonged exposure to sunlight, including transport to the deployment site in lidded coolers.  Upon arrival at the site, each CESU apparatus to be deployed will be slid into a large



Figure 6. Illustration of CESU hanging apparatus.

plastic bag and lowered into the water.  The bag containing the CESU apparatus will be opened below the water surface by a SCUBA diver, to ensure the cages never come into contact with the surface water layer. The SCUBA diver will attach each apparatus to its piling using heavy duty zip ties.  The distal end of the horizontal rebar will be secured to rebar stakes driven into the seafloor at sufficient angles to create a cradle for the horizontal rebar (Figure 6).  This intersection will be secured with heavy-duty zip ties.  CESUs will be installed with the cage bottom approximately 5 cm above the sea floor.  The cage ID number, location of the stake and date/time of deployment will be recorded on the vessel.  Three randomly selected CESUs will be retained for the pre-deployment (initial condition) control samples. The
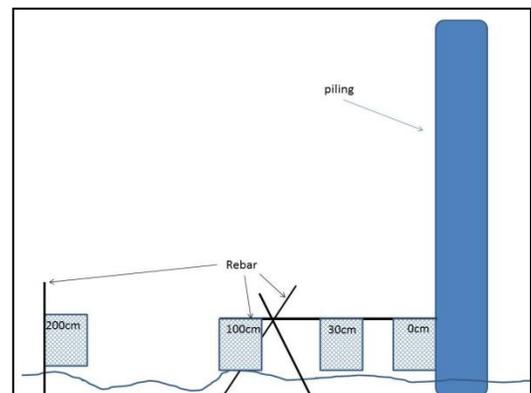
three reference site CESUs will be affixed to single rebar stakes, which will be driven into the seafloor by divers, at the same distance from the seafloor as the CESUs in the piling field.

### 8.1.5 Retrieving CESUs

CESUs will be retrieved between 8 and 10 days after fertilization (7 to 9 days of exposure) to maximize exposure time, while ensuring embryos are retrieved prior to hatching. The steps for cage retrieval will happen in the reverse of the deployment.   A SCUBA diver will detach each apparatus from its piling and place it in a plastic bag.  The diver will hand each bagged CESU apparatus to the boat crew who will place the apparatus into a cooler for transfer to the laboratory.   Once in the lab, CESUs will be detached from the rebar, and each Nitex mesh patch will be removed and placed into a pre-labeled Ziploc bag.   Bagged and labeled Nitex sections will then be placed into a refrigerator or cooler with ice until final processing.

### 8.1.6 Sample identification

CESUs will be identified with sturdy preprinted plastic tags and a field diagram showing relative placement of CESUs by identification tag will be created.8.1.7 Field log

The lead scientist for each field survey will maintain a spiral bound Rite-in-the-Rain field log with detailed notes for each day's activities.  Entries are made in the daily log either in permanent ink or pencil. Minimum information recorded is:

- Name and location of project
- Field personnel
- Sequence of events
- Any changes to plan
- Weather conditions
- Date, time, location name and/or coordinates,
- ID and description of each sample
- Water depth, temperature and salinity
- Unusual circumstances that may affect interpretation of results

## 8.2 Lab Measurements and Standard Operating Procedures

### 8.2.1 Processing CESUs for egg collection

Using clean forceps the Nitex mesh will be carefully removed from its bag and placed egg-side up on a pre-cleaned aluminum foil surface. Two small sub-sections will be removed; (a) a small section of Nitex mesh containing approximately 100 embryos will cut from the main section and re-placed into its labeled Ziploc bag to await mortality assessment, and (b) a second patch of approximately 100 embryos will be cut from the Nitex and placed in a 2 oz. jar containing Stockard's solution, for estimation of developmental stage. Mortality assessments will be made within 24 hours of retrieval, and will consist of counting the number of dead and living embryos from each Nitex patch. Developmental stage will be estimated using an unpublished key developed by WDFW staff (Figure 6).

Embryos will then be transferred from the main Nitex section to sample jars for chemical analysis. The eggs will be scraped off the Nitex mesh using a pre-cleaned stainless steel spatula. The collected eggs will then be transferred to a single 2-oz. I-Chem Series 200



Figure 2. Embryonic developmental stages of Pacific herring (*Clupea harengus pallasi*) (Daniel Penttila, WDF, unpublished data).

**Figure 7. Developmental series for Pacific herring (*Clupea pallasi*)**

composite jar. The weight of the eggs added to the composite jar will be determined by taring the scale to the jar weight prior to adding the eggs. Subsamples from each composite may then be removed and distributed to additional labeled jars or vials for archiving. Samples will be labeled and frozen to -20°C until transfer to the analytical lab. A minimum of 3 grams of tissue will be taken for each sample. When possible, replicate samples will be created for archive.

Because herring embryos are particularly difficult to homogenize using conventional grinding methods, a freeze-thaw cycle will be used to fracture eggs for homogenization. Jars will be placed into a -80°C. freezer for at least 24 hours, then thawed, then repeated. After two or three freeze-thaw cycles thawed eggs can then be simply be mixed with a spatula in its original jar.

### 8.2.2 Mortality Estimates

A small section of Nitex mesh containing approximately 100 embryos will be cut with scissors from each CESU, and re-placed into its labeled Ziploc bag. The number of dead and total embryos on the section will then be counted using visual inspection with a dissecting scope. After mortality counts are made on fresh samples they will be placed in Stockard's solution.
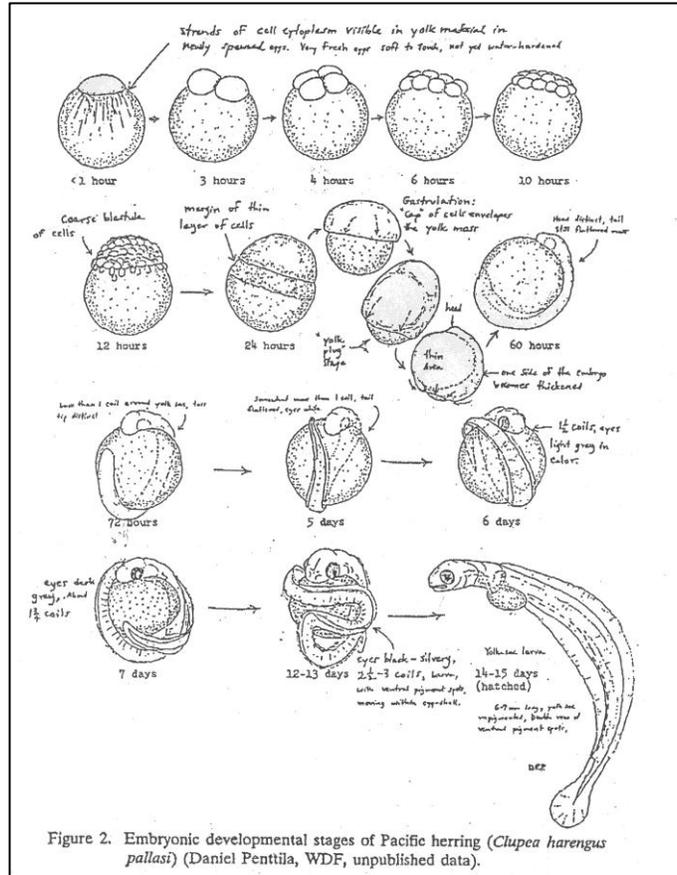
### 8.2.3 Equipment, reagents and supplies for analytical chemistry

Terg-A-Zyme®
Deionized (DI) Water - teflon squeeze bottles
Isopropyl Alcohol - B&J Brand® Multipurpose ACS, HPLC
Tap water
Teflon Squeeze bottles
Heavy duty aluminum foil – Reynolds 627 (60.96 cm wide x 0.94 mm thick)
Scissors - stainless steel
Forceps - stainless steel
Spatula – stainless steel, flat blade/round blade
Mixing spoon – stainless steel
Measuring tape – cloth
Stainless Steel mixing bowl
Sample jars – clear, short, wide mouth 8 oz jars, I-CHEM Certified 200-0250 series, Type III glass with Teflon-lined polypropylene lid  (Figure 7)
Bench scales– such as A&D EK-6000H (6,000 x 0.1 grams) (Figure 8)
Sample jar labels – cryogenic, laser printer ready, Diversified Biotech LCRY-2380 0.94in. x 0.50in and LCRY-1258 2.625in x 1.0in.
Lab coat/apron
Nitrile exam gloves – talc-free
Eye protection
Freezers – walk-in freezer at -20°C, chest freezer at -15°C

### 8.2.4 Lab setup and preparation for tissue chemistry

#### 8.2.4.1 Preparation of Lab Record forms

Specimen forms will be created for this study that will identify samples using nomenclature described below.   A daily log of operations is kept in the lab.  A series of codes are assigned and printed on all lab forms; identification code for the survey (SurveyID), station StationID, specimen (FishID) and sample (SampleID).



**Figure 8.  Pre-cleaned Series 200 I-Chem jar**

#### 8.2.4.2 Use and creation of sampling codes

**SurveyID**:   Each survey carried out by the PSEMP unit is assigned a SurveyID to differentiate it from surveys of the past and future.  The PSEMP database manager creates a unique alpha numeric code that identifies the survey type and the year.



**Figure 9. Bench scale**

**StationID**:  Each station sampled by PSEMP is assigned a StationID code to help differentiate it from other locations sampled in the past, present and future.  The database manager compares the latitude/longitude information for the sampling location in question against those of StationIDs listed in the database to determine if the location has been sampled in the past.  A new location is assigned a descriptive name
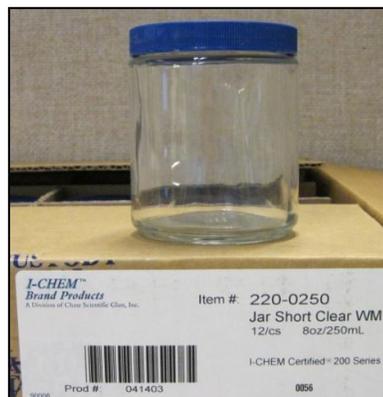
that is unique from all other StationIDs (using all capital letters for the text in the code) and a location which has been sampled in the past is assigned the same SampleID as the past sampling effort(s).

For specimens acquired from a source outside PSEMP (e.g. WDFW test fishery, WDFW survey, Tribal test fishery), if derived from a fixed[2] site, PSEMP uses the sources assigned name as the StationID; however, if the fixed site corresponds to an establish PSEMP station, the PSEMP StationID is used.

**SampleID**:  All samples created by PSEMP are assigned a unique SampleID code that differentiates each sample from similar samples collected in the past, present or future.  A SampleID is a unique alpha-numeric code that is assigned to an analytical sample; either a sample taken from an individual or a composite of individual tissues.  Each id consists of six parts, a two-character year code, a two or more character site code, a dash, a two-character species code, a one or two-character matrix code and either a two-digit (composite sample) or 4-digit (individual FishID) sample number.

Unique SampleIDs are assigned by concatenating numbers of label acronyms as follows:

- Two digit year,
- Two or three (typically) digit station identifier
- A dash "-"
- Two digit species
- Single digit matrix
- A sequential number

  For example :  **13QB-PHSE01**, from 20**13**, **Q**uilcene **B**ay, **P**acific **H**erring, **S**pawned **E**ggs, 01.

## *8.2.4.3 Use and creation of forms*
Once the database manager has determined the sampling codes, he/she then prepares a  Specimen Forms  for use in the lab.  The forms are printed on waterproof paper to facilitate use in the lab environment.  The following information is captured on a Specimen Form:

1. Station Information
    a. SurveyID – database manager provides, preprinted on form
    b. StationID – database manager provides, preprinted on form
    c. Collection Date – preprinted on form and Time?
2. Specimen Information
    a. Species – preprinted on form
    b. Effort – Enter the EffortID if one has been assigned or a general description of the effort (e.g. Tow-1, Tow-2, Set-1, Set-2, Etc.)
    c. CESU ID code
    d. Number of dead embryos from Nitex subsample patch
    e. Total number of embryos from Nitex subsample patch
    f. weight of the mass of embryos placed into the jar in grams
    g. SampleID – database manager provides, preprinted on the form.

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[2] fixed site – a specific location that is returned to repeatedly over time.

3. Observations : comments re: embryo condition including coloration.

### 8.2.4.4 Labeling sample jars

To facilitate identification of composite samples compiled in glass jars, corresponding labels are attached to both the lid and the jar. Both labels are printed on cryogenic, laser printer ready labels produced by Diversified Biotech. The lid label has the SampleID printed on it and the jar label has the Year, Station, Species, Matrix, SampleID, Date (capture), jar Weight (empty weight with lid on) and tissue weight.

### 8.2.4.5 Chain of Custody

A Chain of Custody/Task Order form will be initiated when sample jars are created, to track location, disposition, and entity responsible for each jar. COC forms will be signed and dated each time sample jars change hands, most importantly when they are delivered from WDFW to the analytical laboratory.

### 8.2.4.5 Equipment cleaning procedure

When processing specimens for contaminant analysis, anything (work-surfaces, instruments, etc.) that may contact those portions of a specimen that are subject to contaminant analysis must be cleaned before use.

A "clean" work-surface, means a surface (lab counter, 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.

"Clean" instruments means stainless steel dissection tools and grinding apparatus (hand grinder and cutting blades) that have been washed in warm soapy water (Terg-A-Zyme®), thoroughly rinsed three times under warm running tap water, followed by a rinse with deionized water (held in teflon squeeze bottle), solvent rinsed using isopropyl alcohol (held in a teflon squeeze bottle) and then placed on aluminum foil for air drying.

The same clean instruments/surface can be used repeatedly, without re-cleaning, on specimens contributing to the same composite. They must be subjected to the complete cleaning procedure between composites. Lab personnel must change nitrile gloves between composites.

# 9.0   Chemical Analyses

## 9.1 Analytes

Approximately 38 PAH compounds will be quantitated in this study, comprising 18 low molecular weight compounds and  20 high molecular weight compounds (Table 5).  Nineteen analytes are parent PAH compounds and 19 analytes are alkylated homologs, identified in Tables 5 as "$C_X$-xxx".   In addition, two conventional analytes, total extractible lipids, and percent solids will be measured, all according to Sloan et al. 2004.

**Table 5. Organic compounds to be measured in this study.**

| Persistent organic pollutants: | No. Analytes | Method | Limit of Quantitation - LOQ (wet weight) | Expected Range (wet weight) |
|---|---|---|---|---|
| Polycyclic Aromatic Hydrocarbons (PAHs) | 38 | Sloan et al. 2004 | 0.2-0.8 ng/g | LOQ to 20 ng/g |

[a]  Sloan, C. A., D. W. Brown, et al. (2004). Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants., U.S. Dept. Commerce. NOAA Tech. Memo. NMFS-NWFSC-59.

| Low Molecular Weight | High Molecular Weight |
|---|---|
| acenaphthylene (ACY) | fluoranthene (FLA) |
| acenaphthene (ACE) | pyrene (PYR) |
| fluorene (FLU) | $C_1$-F/P |
| $C_1$-Fluorene | $C_2$-F/P |
| $C_2$-Fluorene | $C_3$-F/P |
| $C_3$-Fluorene | $C_4$-F/P |
| dibenzothiophene (DBT) | benzo[a]anthracene (BAA) |
| $C_1$-dibenzothiophene | chrysene† (CHR) |
| $C_2$-dibenzothiophene | $C_1$-chrysene |
| $C_3$-dibenzothiophene | $C_2$-chrysene |
| $C_4$-dibenzothiophene | $C_3$-chrysene |
| anthracene (ANT) | $C_4$-chrysene |
| phenanthrene (PHN) | benzo[b]fluoranthene (BBF) |
| $C_1$-P/A | benzo[k]fluoranthene†† BKF) |
| $C_2$-P/A | benzo[e]perylene (BEP) |
| $C_3$-P/A | benzo[a]pyrene (BAP) |
| $C_4$-P/A | perylene (PER) |
| retene* | indeno-pyrene (IDP) |
| | dibenzoanthracene (DBA) |
| | benzo[z]pyrene (BZP) |

†coelutes with triphenylene
††coelutes with benzo[j]fluoranthene

## 9.2 Matrix

Herring embryos and wood fragments from CTPs are the two targets of this study.

### 9.2.1 Number of samples

The maximum number of samples to be submitted for chemical analysis in this study is expected to be 52, comprising 29 samples prior to piling removal and 23 samples after piling removal.

### 9.2.2 Analytical methods

All PAHs in this study will be analyzed according to Sloan et al. (2004), to provide consistency with previous WDFW/PSEMP studies.  In brief, this method comprises three steps:  (a) extraction, (b), cleanup by silica/aluminum columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and (c) quantitation of chlorinated hydrocarbons (CHs) and aromatic hydrocarbons (AHs) using gas chromatography /mass spectrometry (GC/MS) with selected-ion monitoring (SIM).  Samples are extracted using accelerated solvent extraction (ASE with methylene chloride), which provides an extract that can be used for AH, CH recovery and gravimetric lipid evaluation.  This method also includes alterations to typical GC/MS methods to stabilize the instrument and improve accuracy such as chemical ionization filaments (to increase source temperature), employing a cool on-column injection system in the GC, a guard column before the analytical column, and point-to-point calibration to improve data fit over the full range of GC/MS calibration standards (Sloane et al. 2004).

### 9.2.3 Sensitivity/Method Detection Limit (MDL)

The Lower Limit of Quantitation (LOQ) for all PAHs in this study is "the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration.   When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ."  (Sloan et al. 2006).  Typically LOQ values for POPs that have been reported to PSEMP by this method are in the range of 0.2 to 0.8 ng/g wet weight of original tissue (in this case homogenized embryos).

## 9.3 Sample preparation methods

Eggs samples are homogenized in the per Section 8.2.1.  Prior to extraction each homogenized sample should again be mixed thoroughly with a clean spatula or other utensil.  Wood fragments will be macerated with a heavy cleaver prior to extraction

# 10.0     Quality Control Procedures

Quality control of all field activities will be supervised by the PI.  All personnel will have available to them copies of the QAPP and pertinent SOPs.  The PI will review all notes entered into the field log at the end of each activity, and prior to leaving each study site or other significant location.  For analytical chemistry, quality control procedures, quality assurance criteria and corrective actions for persistent organic pollutant (POPs) data are detailed in Sloan et al. (2006).  Briefly, precision is monitored and controlled within batches using laboratory replicates of field samples (2 replicates run for every batch of 12 samples) and across batches by analyzing Standard Reference Materials (SRMs – one per batch).  Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements.  The RSD of analyte responses relative to the internal standard must be ≤ 15% for the repetitions.

For POPs analysis, accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values for 1974b Blue Mussel.  Concentrations of ≥70% of individual analytes are to be within 30 % of either end of the 95% confidence interval of the reference values.  One method blank is run for every 20 or fewer field samples. No more than 5 analytes in a method blank are to exceed 2x the lower LOQ before corrective action is taken.  The corrective action will be to re-extract and re-analyze the affected samples.   Data are reported by the analytical lab without blank correction.  It is up to the user to decide if and how to correct data with respect to blank contamination, and how or whether such data should be censored with qualifiers.  At least one internal standard (surrogate) is added to each sample, with acceptable recoveries ranging from 60 to 130%.

# 11.0     Data Management Procedures

## 11.1 Data recording/reporting requirements

Data for both field samples and QC samples are received from analytical laboratories in Excel spreadsheets in various formats.  PSEMP staff format these data into a structure compatible with the Toxics in Biota (TIB) database.  The TIB database is a relational format created in Access, with separate tables for (1) field effort data, (2) biological characteristics of individuals used to create samples, (3) many-to-many cross reference for individuals-to-composites, (4) sample tracking, condition  and summary statistics,  and (5) chemical analyses.  Data are examined visually using Excel filters and sorting procedures to identify gross formatting or transcription errors.  Raw analyte concentrations are compared with expected ranges to identify potential outliers.  In addition preliminary summary statistic tables, scatter plots, and time trend plots are created to examine the new data.

## 11.2  EIM data upload procedures

All data generated by this project will be submitted to Ecology's EIM for later export to EPA's STORET database.

# 12.0    Audits and Reports

## 12.1 Frequency of Audits

The NWFSC analytical lab participates in annual NIST or IAEA interlab comparison studies.

## 12.2 Responsibility for reports

WDFW staff will submit a draft report with the following outline to peer reviewers and to the NEP QC for comment.

Bulleted OUTLINE

The final report will address comments received as deemed appropriate.  Data packages will be prepared for submittal to EIM and later export to EPA's STORET database, as detailed in the Scope of Work.  James E. West is responsible for these products.

# 13.0    Data Verification and Validation

## 13.1 Field data verification, requirements, and responsibilities

All sample location data for this study will be verified by comparing GIS-plotted latitude and longitude data with field notes.

## 13.2 Lab data verification and validation

Data generated by the analytical lab are reviewed for out-of-bounds values, transcription errors and other problems by at least two chemists.  Final review is conducted by a lab manager who approves data before they are released to the client. Prior to database entry WDFW staff will compare results with MQCs and review data by comparing results with similar species or matrices in the PSEMP database. Individual data, means, and standard deviations are plotted and putative outliers evaluated for validity. Evaluation of the validity of putative outliers includes reviewing all collection, biological, and analytical data for potential transcription errors, communication with analytical labs to verify reported values are correct, and evaluation of biological covariates that might explain otherwise unanticipated values. PSEMP does not currently conduct data validation by a third party reviewer.

# 14.0    Data Quality (Usability) Assessment

## 14.1 Process for determining whether project objectives have been met

The success of meeting data quality objectives is evaluated based on the outcome of quality control procedures during analytical procedures.  Typically if QC criteria are not met the problem is identified by staff from the analytical lab, corrected, and sample (or extract) re-run.  In cases where QC criteria have not been met and there is not enough tissue to be reanalyzed, the data will be censored with appropriate qualifiers to allow an objective evaluation of the usability of the final record.  Rejected data are censored with an "R" or equivalent qualifier.  We expect rejected data to be rare based on (1) a long history of employing these methods to measure target analytes in a wide range of Puget Sound biota matrices, (2) the range of data values we expect in this study, and (3) appropriate (tenth-of-ppb) limits of quantitation ( with the singular possible exception of potential blank contamination for naphthalene-compounds).

Adequacy of sample number will be evaluated during the statistical analysis of analytes.  We have predicted that three to five replicates per class will provide enough power to distinguish spatial trends in most individual PAH analytes, however a final evaluation of sample size adequacy will be made after this analysis.

## 14.2 Data analysis and presentation methods

Toxics data collected for this study are part of a long-running tissue residue monitoring program.  This program has a long history of data analysis and presentation, which will be continued in the present study.  Analysis and presentation of contaminant and covariate data will be conducted using programs commonly employed by PSEMP to compare spatial distribution of contaminants.  This includes a General Linear Model that compares contaminant concentrations across geographic locations while adjusting for potentially confounding  covariates such as animal size and age.  In this study covariates are controlled by study design, so analyses will be conducted using simple ANOVA to compare populations.  PAH results may be log-normalized to achieve normality and homoscedasticity.  A Tukey's *post hoc* multiple range test will be used to discriminate the significance of observed differences by sample type and between sampling periods (before *vs* after CTP removal).  Linear regression may be used to test the significance of PAH gradients with distance from pilings.   If normality and homoscedasticity are not achievable with data transformation, non parametric analogs of ANOVA may be used.  Similarity matrices of individual PAH analytes will be created to perform Multivariate Dimensional Scaling comparisons among sample types, and used to compare PAH patterns. This latter method will be used in particular to compare PAH patterns in embryos with patterns measured in wood from the CTPs.

We will compare tissue residues of PAHs in herring embryos (eggs) with published PAH effects thresholds such as those described by Carls et al. (1999). These authors reported a range of sublethal and lethal effects including yolk sac edema and premature hatching in herring embryos exposed to dissolved PAHs, resulting in tissue total PAH concentrations ranging from 22 to 108 ng/g wet weight.

## 14.3 Treatment of non-detects

Non detected analytes are censored with a "less than limit of quantitation" (<LOQ) or "U" qualifier.  The value reported for non-detected analytes will be the limit of quantitation.  It is the responsibility of users to decide how to use censored data.

## 15.0　　　References

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