# Effectiveness Monitoring for a Creosote-Piling Removal Project: Embryos of Pacific Herring (*Clupea pallasii*) as Sentinels for the Presence of Polycyclic Aromatic Hydrocarbons (PAHs)

By James E. West, Andrea J. Carey, Jennifer A. Lanksbury, Laurie A. Niewolny and Sandra M. O'Neill

01 February, 2016

Washington Department of
FISH and WILDLIFE

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## Author and Contact Information

James E. West (corresponding author)
James.West@dfw.wa.gov
(360) 902-2842

Andrea J. Carey
Andrea.Carey@dfw.wa.gov
(360) 902-2710

Jennifer A. Lanksbury
Jennifer.Lanksbury@dfw.wa.gov
(360) 902-2820

Laurie A. Niewolny
Laurie.Niewolny@dfw.wa.gov
(360) 902-2687

Sandra M. O'Neill
Sandra.Oneill@dfw.wa.gov
(360) 902-2666

Marine Resources Division
Washington Department of Fish and Wildlife
600 Capital Way N
Olympia, WA 98501-1051

Funding for this study was provided in part by the United States Environmental Protection Agency (EPA) National Estuary Program (NEP), under Puget Sound Ecosystem Restoration and Protection Cooperative Agreement grant (G1200469) with Washington Department of Ecology.

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# EXECUTIVE SUMMARY

This study was originally designed to evaluate the degree to which removal of creosote-treated pilings (CTPs) would remove the risk of exposure of biota to creosote related toxic chemicals in Puget Sound. To that end, WDNR contracted with WDFW staff from 2013 to 2015 to use monitoring tools used or developed by WDFW in their toxic monitoring program under the Puget Sound Ecosystem Monitoring Program (PSEMP) to evaluate the effectiveness of the CTP removal procedure in one of the several piling fields designated for removal in 2013. The piling field selected for the study, a derelict railroad trestle in Quilcene Bay, was ideal for the study in many ways, including its small size (which reduced the need for spatially extensive sampling), and its isolation from other (non-CTP) sources of chemical contamination that might otherwise have confounded interpretation of the putative CTP chemical source.

Pacific herring were selected as the indicator species for this study because they have historically used Quilcene Bay for spawning, including on eelgrass beds at the Quilcene CTP field, and developing fish embryos are known to be particularly susceptible to many of the chemicals known to occur in the creosote used to treat wood pilings. The pre-removal part of this study identified and described the contaminant risk faced by herring embryos spawned in close proximity to derelict, approximately 100-year-old CTPs. A suite of 42 individual chemicals in a class of polycyclic aromatic hydrocarbons (PAHs), which are typically abundant in creosote, were measured in embryos developing within two meters of derelict pilings. Mortality of embryos increased with increasing PAH levels, even though the PAH levels were relatively low compared with other published studies, suggesting that even old derelict pilings can pose a risk to embryos developing near them.

The original intent of the study was to compare PAHs in herring embryos in a CTP field prior to piling removal with embryos in the former CTP field area one year after pilings had been removed. However, this intent was never fulfilled because CTPs in Quilcene Bay were never fully removed according to the project plan and Best Management Practices; many CTPs within the study area were cut off at or above the seafloor and significant piling/wood debris was left behind, resulting in new and worse PAH conditions. PAH levels in 2014 embryos (2 weeks after removal activities) and 2015 embryos (one year later) were 17x greater than 2013 embryos prior to the removal.

The area affected by PAH contamination was small relative to the total area Pacific herring typically use for spawning in Quilcene Bay, and the actual area used by Quilcene herring in any given year is unpredictable. Hence, the risk to herring from CTP contamination in any given year is difficult to estimate. The unique circumstances that occurred at this site regarding the high percentage of pilings that broke and could not be fully extracted (resulting in the terms for the CTP removal contract not being fulfilled) will require additional study and cleanup efforts to reduce potential impacts to herring embryos.

# INTRODUCTION

The study presented here was designed to evaluate the effectiveness of removing derelict creosote-treated pilings (CTP) in reducing exposure of organisms to creosote-related toxic contaminants in Puget Sound nearshore habitats. The study took place at a single CTP field in Quilcene Bay, Washington, which was constructed as a railroad trestle around 1925, and which is located in an area historically utilized by Pacific herring (*Clupea pallasii*) for spawning (Figure 1). The study was conducted over a three year period, from 2013 through 2015, and it used Pacific herring embryos as an indicator to measure changes in CTP-related contaminants related to CTP removal.

The study area contained the remains of a railroad trestle, which comprised more than 300 CTPs, originally constructed sometime between 1891 and 1925 to accommodate transferring materials from ship-to-shore along a shoreline otherwise lacking a deep-draft harbor (Christopherson, 2012). During its approximately 100 year presence in the bay, the trestle may have represented a local source of creosote-related contaminants to its nearshore organisms. It was one of several CTP fields identified by the Washington Department of Natural Resources for removal in 2013. The isolation of the Quilcene CTP trestle was advantageous because it facilitated discerning CTP contaminant sources from other potential sources. Aside from the possibility of contamination near the CTP trestle, Quilcene Bay was considered the least contaminated of five herring spawning areas in a Puget Sound-wide survey in 2001 (West et al. 2014). Moreover, there is

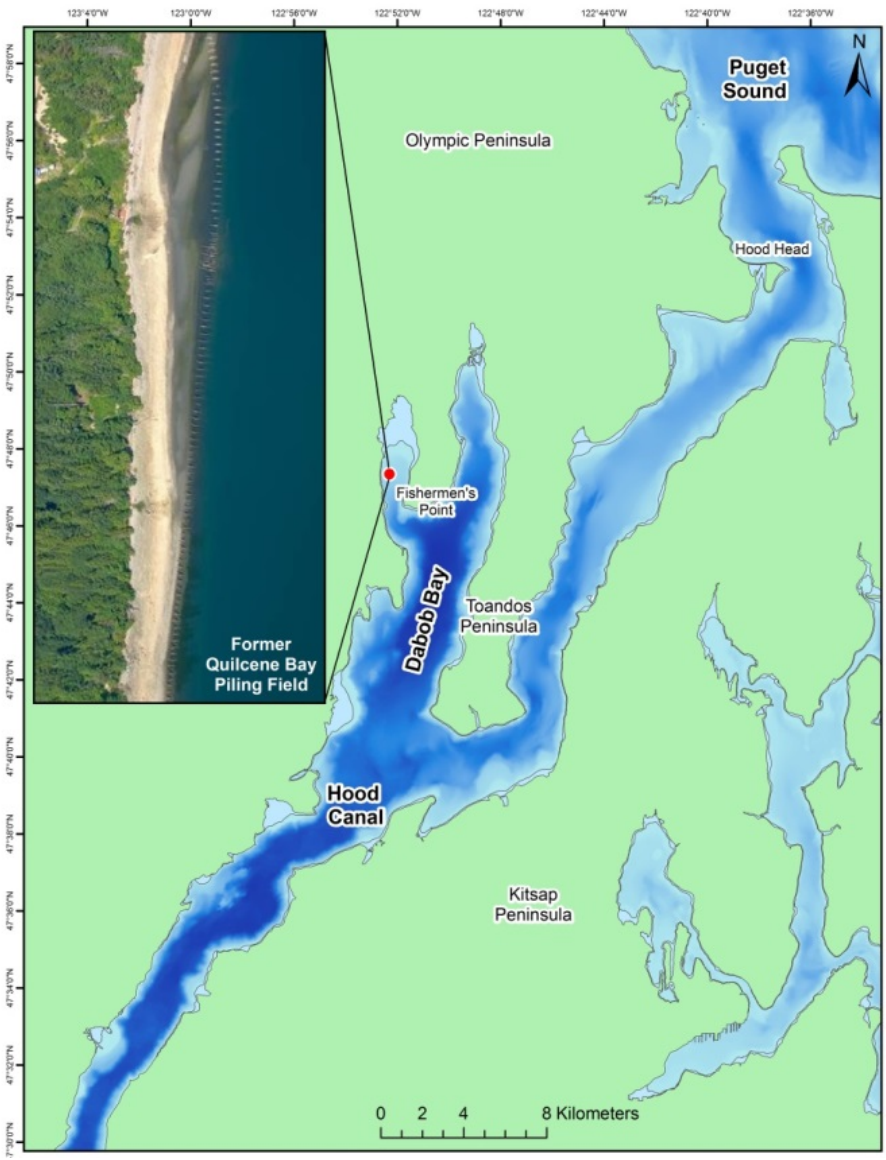


**Figure 1. Map of Quilcene Bay and the surrounding area. The red dot indicates the location of the**

little shoreline development or other obvious sources of chemical contaminants in Quilcene Bay, except for a small marina and aquaculture facility approximately 500 m to the north of the trestle, and shellfish growing rafts approximately 2km to the southeast.

Pacific herring are a common and abundant, small-bodied, schooling, pelagic planktivore that spawn on nearshore vegetation throughout Puget Sound. Developing embryos of fish such as herring and other nearshore spawners are particularly sensitive to low concentrations of PAHs they may be exposed to in their incubation habitats (Carls et al., 1999; Heintz et al., 1999). West et al. (2014) recently reported PAH uptake in herring embryos from several locations in Puget Sound, implicating PAHs as a potential risk to herring embryo health. Other lab and field studies have demonstrated links between aqueous PAHs and sublethal effects in fish embryos including cardiac edema and arrhythmia (Incardona et al., 2009; Incardona et al., 2004). Cardiac toxicity was reported in herring embryos exposed to oil from the 2007 Cosco Busan oil spill in San Francisco Bay (Incardona et al., 2012). These authors also reported mortality and tissue necrosis related to background pyrogenic[1] PAHs. PAH exposure of embryos from creosote-treated pilings has also been identified as a potential source of mortality in developing herring embryos. A study by Vines et al. (2000) reported high mortality in herring embryos spawned on creosote-treated pilings. Toxicological effects of PAHs on embryo health can also be exacerbated with addition of ultraviolet light from sunlight (Barron et al., 2003; Barron et al., 2005; Hatlen et al., 2010), so embryos spawned in shallow waters may be particularly susceptible to the combination of PAHs and sunlight.

This report documents and summarizes PAHs and mortality measured in herring embryos that were manually spawned, deployed, and incubated *in situ* in close proximity to the Quilcene Bay CTP trestle. The study was originally designed to sample herring embryos deployed at the Quilcene CTP trestle approximately six months before and six months after CTP removal. Embryos were sampled in the winter of 2013[2] to document the "before removal" condition; CTP removal was scheduled for July 2013, and the "after removal" sampling scheduled for the winter of 2014. However, the CTP removal was delayed by seven months, which resulted in the winter 2014 sampling being conducted only two weeks after the final CTPs were removed from the Quilcene Bay trestle area. For that reason, the 2014 sampling was considered a "during removal" sample, and a third effort was added in the winter of 2015, one-year "after removal". In addition, the CTP removal process was incomplete; a number of pilings were cut at or above the mud-line, and substantial CTP debris remained on the seafloor within the sampling area. The CTP debris remained at the trestle site through 2015, which prevented a realistic evaluation of what would otherwise have been considered after-removal conditions. Many of the 2014 and 2015 embryo cages were deployed in close proximity or contact with CTP debris. Because of this, the study's original intent of measuring an "after removal" condition was not fulfilled.

[1] PAHs resulting from the combustion of organic matter, especially fossil fuels

[2] Field sampling was only possible during herring spawning season, from roughly late January through early March

## Methods and QAPP Compliance

Sampling and analytical methods in this study followed standard operating procedures detailed in the Quality Assurance Project Plan (QAPP) written for this work (West et al., 2013), and briefly summarized below. This study was designed to investigate the relationship between proximity to creosote pilings and levels of PAHs in herring embryos, and to determine whether the removal of creosote pilings reduced exposure to PAHs for herring embryos developing nearby. To achieve this, manually-spawned herring embryos were placed in proximity to CTPs for a 10–day incubation period; embryos were created by manually fertilizing herring eggs using gametes collected from pre-spawning adults. The highly adhesive eggs were placed on nylon mesh inside protective cages and deployed during the normal spawn-time for Quilcene Bay herring which is late January through early March. For each year of the study, embryos remained *in situ* for 10 days, after which they were retrieved and processed for chemical analyses and mortality. Deployments were conducted in in late March/early April of each of the three years of the study; 2013 (before CTP removal), 2014 (2 weeks after CTP removal), and 2015 (one year after CTP removal).

Overall, the creation and deployment of embryo cages, *in situ* procedures, and analytical methods were completed in close compliance with the QAPP. However, a change in the CTP removal schedule and incomplete removal of CTPs prevented this study from achieving its original goal of measuring "after removal" conditions. As mentioned above, the CTP trestle was to be removed during the summer of 2013 but in fact CTPs were not removed until late winter 2014. The removal project ended only roughly two weeks prior to initiating the second year of sampling, and the CTP removal was incomplete, leaving cut piling ends at or above the mudline, and splintered CTP debris along the study area (Figure 2).

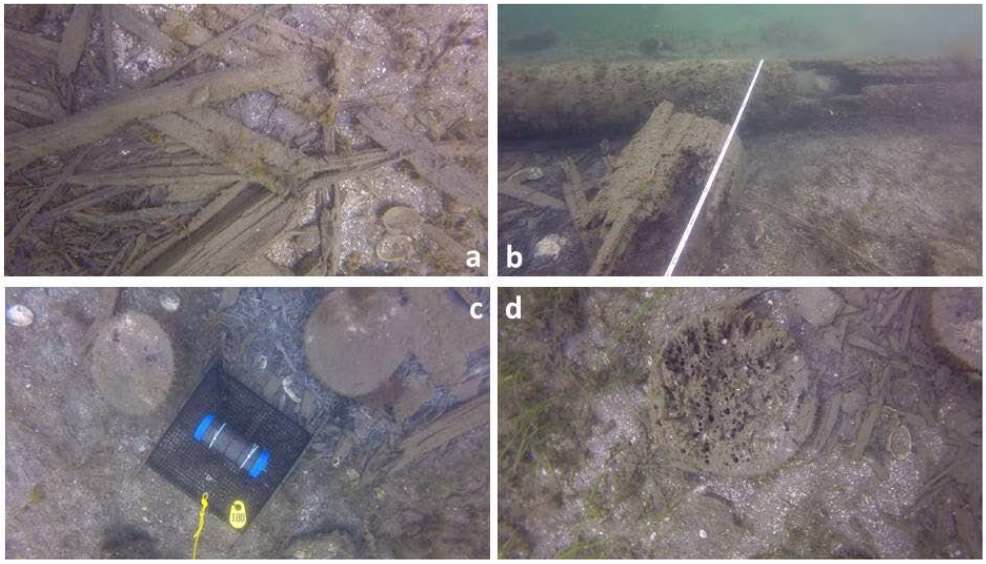


**Figure 2. Creosote-treated piling (CTP) debris on the seafloor in the high density piling area one year after CTP removal: a) CTP splinters, b) cut or broken CTPs, c) a caged embryo sampling unit (cylinder with blue end-caps) on its mesh base, between two pilings that had been cut off at the mud line, and d) a piling that had broken off at the mid line. The cage shown in c) was deployed in 2015 and exhibited the third-greatest total PAH concentration in the study (still photos taken from video shot by Larry LeClair and Taylor Frierson in 2015)**

High winds on the day of deployment in 2014 prevented accurate deployment of caged embryo sampling units (CESUs). Because of this, two CESUs meant to be deployed as "inshore" samples were actually closer to the high density piling area (HDPA). These samples were subsequently re-classified as HDPA for statistical analyses.

The aluminum cages used for the CESUs were difficult to maintain because of oxidation of the metal surfaces after the cages were removed from the field. Soaking and cleaning cages in fresh water failed to prevent significant damage to the aluminum in the months after retrieval, when the cages were being stored in the lab. To overcome this problem we switched from the aluminum cages to cylindrical bait holders constructed of plastic and epoxy-coated mesh (description below). An additional piece of Nitex mesh (with no eggs attached) was used to line the inside of the cage to exclude potential predators.

## Adult Herring Collection

All adult herring for spawning were collected in the nearshore waters at the south end of Bolton Peninsula (Fishermen's Point) near Quilcene Bay (Figure 3). In 2013 approximately 458 herring (285 males and 173 females) were collected from eight gill net hauls during the evening of March 18; in 2014 70 herring (48 males and 22 females) were collected from four gill net hauls during the evening of March 26; in 2015 approximately 200 fish (100 males and 100 females) were collected from two gill net hauls during the evening of March 19 (see Supplemental Table 1 for detailed location information).

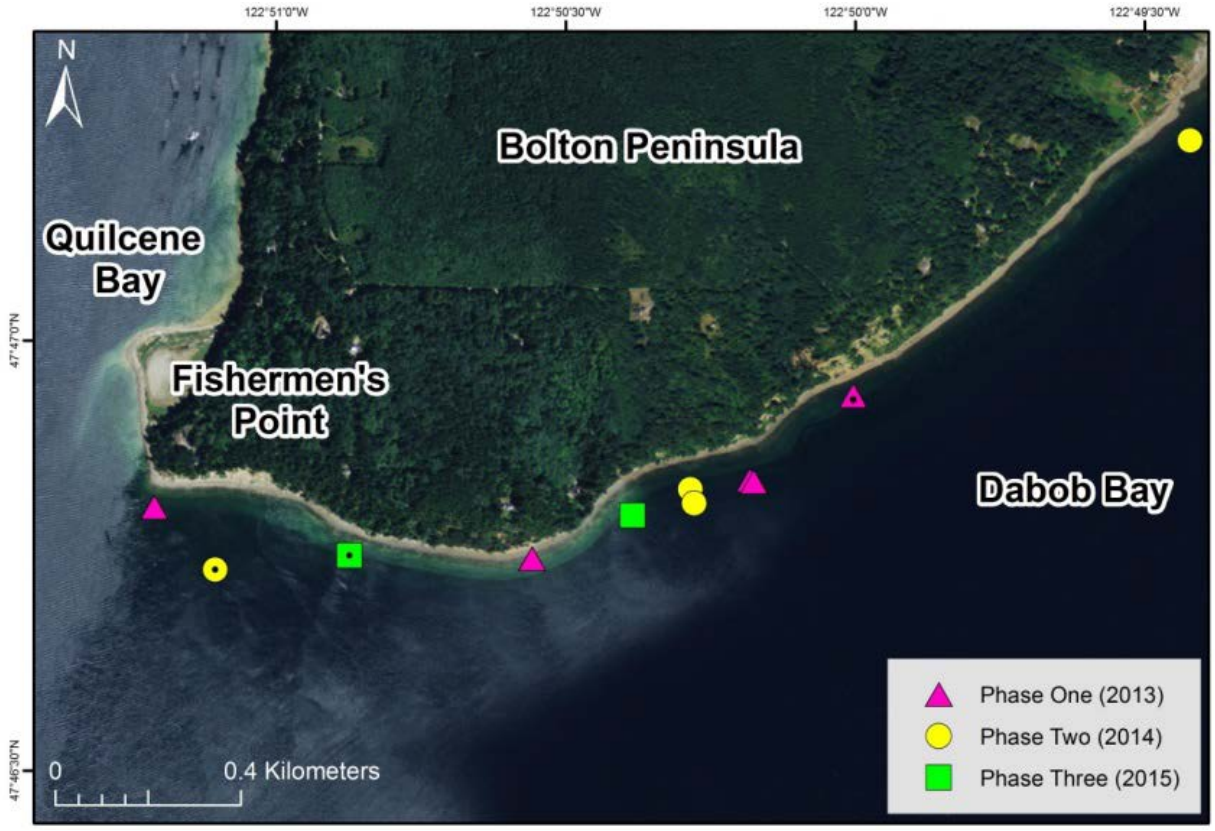


**Figure 3. A map of the adult herring collection locations off Fishermen's Point, Dabob Bay, for 2013, 2014 and 2015. A symbol with a black dot in the center indicates where the majority of the fish used for the study were collected.**

## Manual Fertilizations

In 2013, fertilizations took place at the Troutlodge Aquaculture facility, located adjacent to the former WDFW Point Whitney Laboratory in Brinnon, WA. In 2014 and 2015, fertilizations took place at Environ Laboratories in Port Gamble, WA. Fertilization success was measured in sub-samples of embryos collected from random pieces of mesh panels approximately two hours post fertilization (hpf). In 2013, fertilization success was >60%, and in 2014 and 2015 it was >90%. Development of embryos was examined again, approximately 19-20 hpf, just before mesh panels were processed for deployment, and rates ranged from 56 to 100%.

## Deployment Controls

Three composite samples of unfertilized eggs from ovaries of eight fish each were sampled for PAHs to evaluate the potential transfer of PAHs from mother to egg. In addition, four deployment controls were sampled, each consisting of a sample of embryos that had been carried into the field, placed in a CESU, and handled in every way the same as a deployed CESU, except that the embryos were retained on the day of deployment rather than placed in the water. These deployment controls were then sealed in a Ziploc bag and the embryos sampled later the same day for chemical analyses, to evaluate the potential exposure of embryos to exogenous PAHs during the process of transferring the egg-laden mesh from the fertilization tank to transfer bags, transportation to the deployment site, and insertion into the CESU. Three deployment controls were taken in 2013, and one in 2015 (Table 1).

## Cage Deployment and Retrieval

In 2013, WDFW PSEMP biologists, SCUBA divers from WDFW's Subtidal Shellfish Dive Unit, and a member of the WDFW's Oil Spill Response team deployed 20 CESUs within the high density CTP area (Table 1, Figure 4, and Supplemental Table 2), and three CESUs at the Fishermen's Point reference site (Table 1, Figure 5, and Supplemental Table 2). To deploy CESUs at fixed distances from CTPs, CESUs were affixed with nylon cable ties to two parallel 120-cm lengths of bent-tip steel rebar, at 0 cm, 30 cm, and 100 cm distance from a CTP (Figure 6). The bent rebar ends allowed attachment to pilings with cable ties, with the bars oriented horizontally to, and approximately 10 cm above, the seafloor. The fourth CESU (200 cm) was deployed separately, attached to vertical rebar stakes driven into the substrate, to avoid using unwieldy lengths of horizontal rebar. CESUs remained attached in this manner for 10 days, after which they were retrieved (Table 1). In addition, naturally occurring eggs were collected from *Laminaria* and *Sargassum* at the reference site using an iron rake designed and used by the WDFW Forage Fish Unit for such purpose (Table 1). We also sampled wood chips from the underwater portion of a CTP during the 2013 CESU deployment. These wood chips were placed in a Ziplock bag and brought to the surface, where they were placed in an I-Chem jar for later analysis.

In 2014, PSEMP biologists and a member of the WDFW's Oil Spill Response team deployed 15 CESUs among the former high density piling area (HDPA) and in the inshore (west) and offshore (east) waters adjacent to the HDPA (Table 1; Figure 4). An additional three CESUs were deployed at the Fishermen's Point reference site (Table 1; Figure 5). Because the pilings were absent in 2014, the rebar frames from 2013 were unnecessary. Rather, each 15 x 15 $cm^3$ CESU was attached to an anchor frame consisting of a 41x41x6 cm polyvinyl-coated steel mesh panel, with edges bend downward to suspend CESUs

approximately 10 cm above the seafloor (as in 2013). To ensure upright placement of the CESU on the seafloor and anchoring of the unit in the sediment, two 61-cm pieces of 1.6 cm-diameter steel rebar were attached as ballast to two parallel sides of the anchor frame. Each CESU was deployed with a unique ID number and a line and buoy for retrieval (Figure 7). As in 2013, the 2014 CESUs were retrieved after 10 days of exposure.

**Table 1. Important dates for all years of the study and herring egg sample sizes by cage location including the number of CESUs deployed and the number of samples analyzed for contaminants.**

| Year | Important Dates | Site | # deployed/# analyzed Eggs |
|---|---|---|---|
| 2013 | 3/18/13[a], 3/21/13[b], 3/22 – 4/1/13[c] | Deployment Control | 3/3 |
| | | CESU at Fishermen's Pt. Ref. Site | 3/3 |
| | | Naturally spawned embryos | --/6 |
| | | 0 cm from CTP | 5/5 |
| | | 30 cm from CTP | 5/5 |
| | | 100 cm from CTP | 5/5 |
| | | 200 cm from CTP | 5/5 |
| 2014 | 3/26/14[a], 3/27/14[b], 3/28 – 4/7/14[c] | Deployment Control | 0 |
| | | CESU at Hood Head Ref. Site | 5/5 |
| | | CESU at Fishermen's Pt. Ref. Site | 4/4[a] |
| | | HDPA | 7/7 |
| | | Inshore | 4/4 |
| | | Offshore | 4/4 |
| 2015 | 3/19/15[a], 3/20/15[b], 3/21 – 3/30/15[c] | Deployment Control | 1/1 |
| | | CESU at Fishermen's Pt. Ref. Site | 3/3[a] |
| | | HDPA | 8/8 |
| | | Inshore | 3/3 |
| | | Offshore | 3/3 |
| | | *2013 subtotal* | *26/32* |
| | | *2014 subtotal* | *27/27* |
| | | *2015 subtotal* | *18/18* |
| | | ***Overall Total*** | ***71/77*** |

[a]All embryos deployed at this reference area died – sample not used

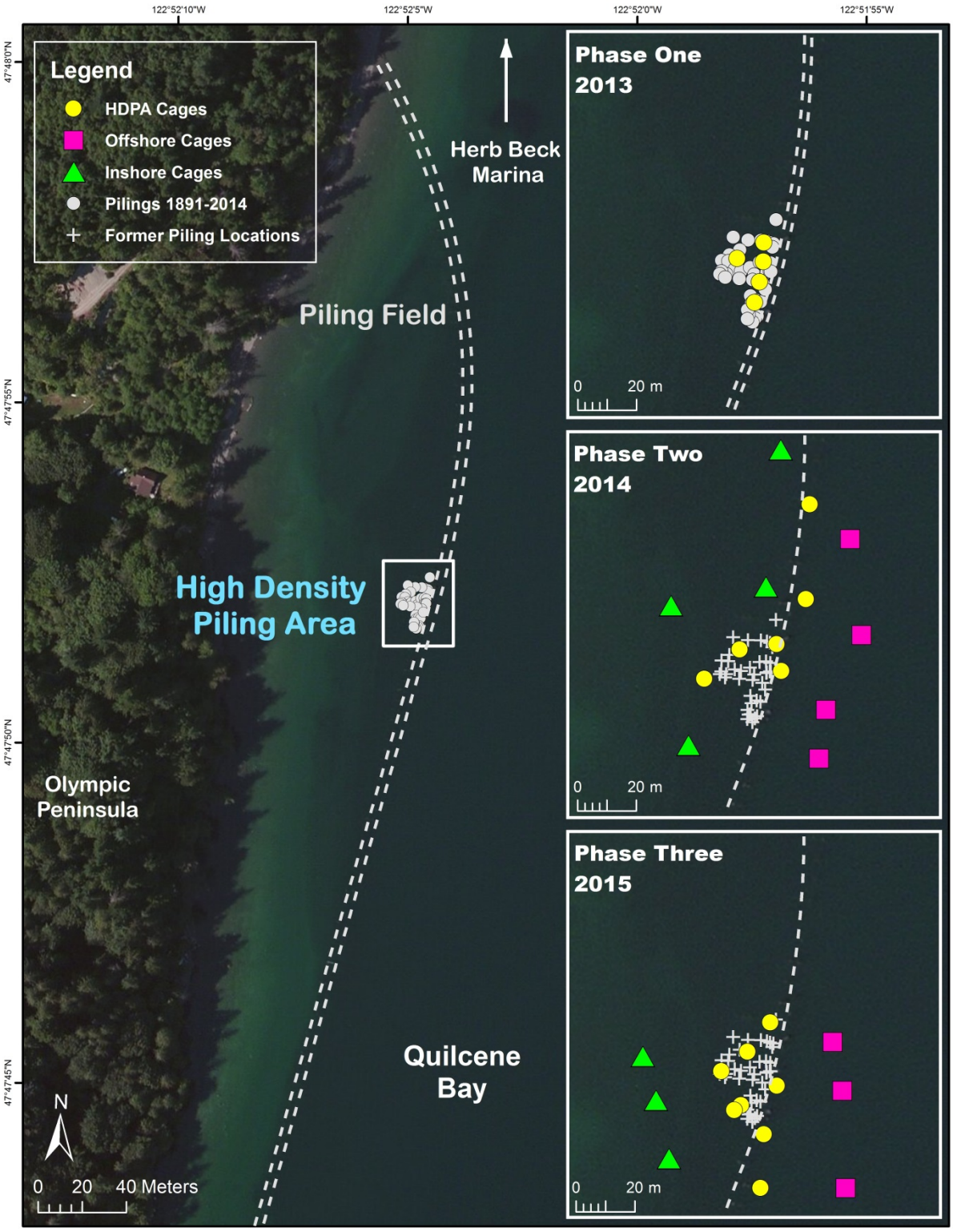


**Figure 4. Map of the study site and CESU locations in Quilcene Bay, WA during all three years of the study. The double gray-dotted lines represent the location of the line of low density pilings and the single gray-dotted line represents the former location of the low density pilings after removal. See Supplemental Table 2 for coordinates of all CESUs deployed.**

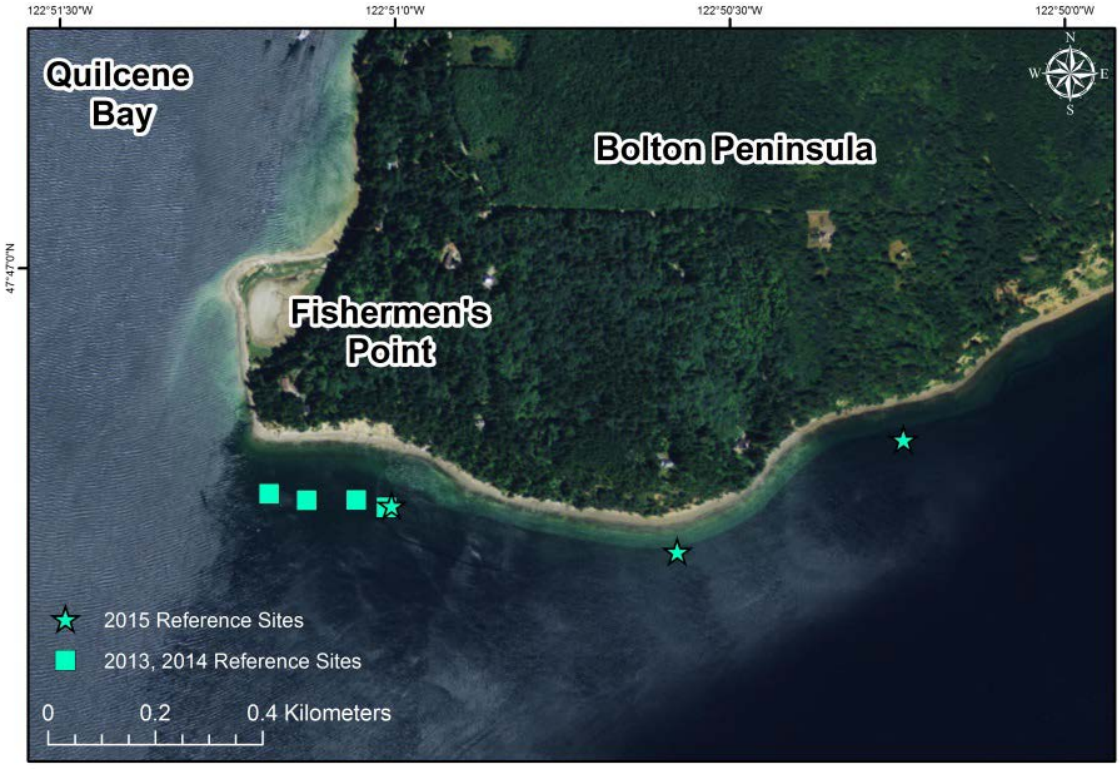


**Figure 5. Map of the reference site cage locations off Fishermen's Point and the Bolton Peninsula in Quilcene Bay during all years of the study. Cage coordinates were estimated in 2013.**

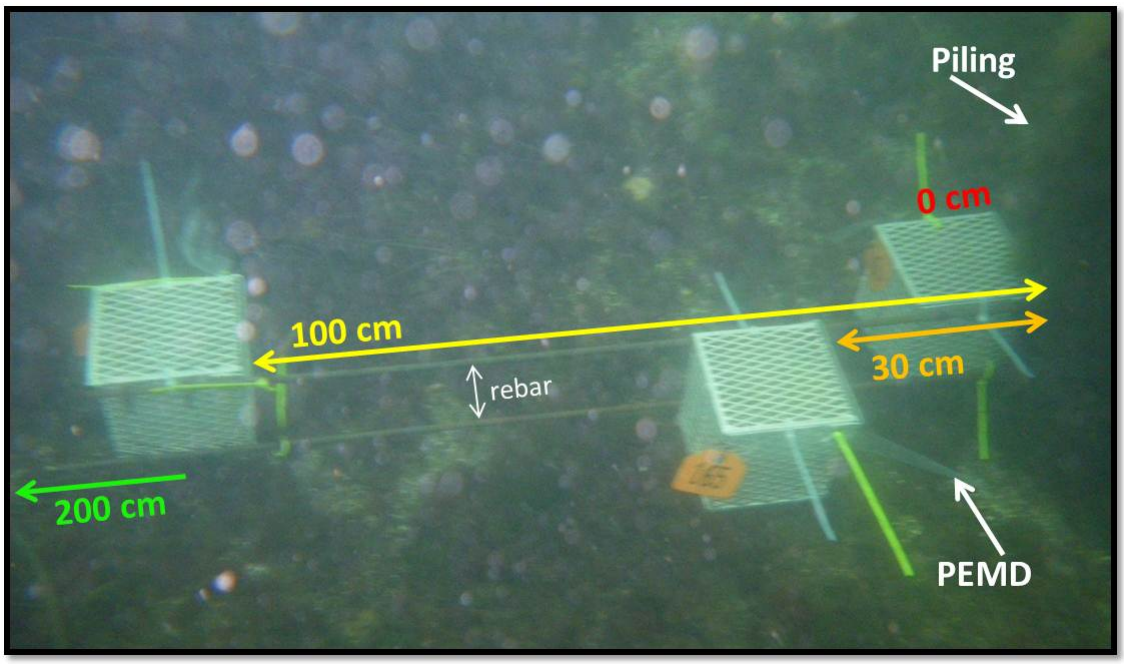


**Figure 6. Caged embryo spawning units (CESU) from the first year of the study (2013).**

In 2015, WDFW PSEMP biologists and a volunteer deployed 14 CESUs in the former HDPA and in the inshore (west) and offshore (east) waters adjacent to the HDPA, at similar locations to the 2014 CESUs (Figure 4). Similar to the previous years, three reference site CESUs were deployed at Fishermen's Point and one deployment control was held back prior to deployment in order to evaluate potential contaminant exposure from CESU transport and deployment procedures (Table 2). All CESUs were retrieved after 10 days of exposure.

**Figure 7. A deployed CESU from the third year of the study (2015).**

## Laboratory Activities

Samples were taken for tissue PAH analysis in all three years by scraping embryos from the mesh into pre-cleaned I-Chem II jars immediately following retrieval. Samples for assessing mortality were taken as a section of embryo-laden mesh preserved in Stockard's solution. Because of the high numbers of embryos involved in the study and the difficulties maintaining adequate incubation conditions for retrieved herring embryos, it was unfeasible to directly conduct hatching success (counts of larvae) on all samples. As a compromise, we counted pre-hatched embryos at retrieval (11 dpf), for a development assessment. Counts were conducted on embryo samples preserved in Stockard's solution. The total number of embryos on a mesh sample that had clearly developed normally to the 11 dpf stage was compared with the number of eggs that had clearly been fertilized but had died. The proportion of dead pre-hatching embryos was calculated from the total of these two embryo types to determine the mortality rate of the embryos to the hatching stage.

## Analysis of Polycyclic Aromatic Hydrocarbons

Forty-two PAH compounds were quantitated in this study, consisting of 22 low molecular weight compounds and 20 high molecular weight compounds (Table 3). All PAHs in this study were analyzed according to Sloan et al. (2014) to provide consistency with previous WDFW/PSEMP studies. In brief, this method comprised three steps: (a) extraction, (b) cleanup by silica/aluminum columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and (c) quantitation of aromatic hydrocarbons (AHs) using gas chromatography/mass spectrometry (GC/MS) with selected-ion monitoring (SIM). Samples were extracted using accelerated solvent extraction (ASE) with methylene chloride, which provided an extract that was used for AH recovery and gravimetric lipid evaluation. The amount of lipids present in the embryos, reported as percent lipid, was measured by the gravimetric method according to Sloan et al. (2004).

In cases where a PAH was not detected in a sample, the value was reported as "less than the limit of quantitation", or "<LOQ". An LOQ value was calculated for each sample based on sample weight and instrument performance for each batch of samples (see Sloan et al. 2014). PAH summations were based on detected values only; a value of zero was substituted for <LOQ for calculating total PAHs (TPAH). In cases where all PAHs were not detected in a sample, a value of 0.25 ng/g wet wt was used as the TPAH concentration.

Some PAH analytes were occasionally detected in solvent-blank samples. In cases where a blank-detected PAH was also detected in a field sample from the same batch, the value of the PAH in the blank sample was subtracted from the value of the field sample if the PAH value of the field sample exceeded five times the value of the blank-sample. If the PAH value of the field sample was less than five times the value of the blank-sample, the value of the PAH in the field sample was considered indistinguishable from the blank (i.e., the PAH originated from post-retrieval contamination), and it was reported as a non-detect (i.e., <LOQ).

## Data Quality

All data met the quality control limits identified in Sloan et al. (2014) and detailed in the QAPP for this study; case narratives in the Supplemental Information section summarize quality assurance for all analyses.

# RESULTS

## TPAH Concentration in Embryos

The following TPAH results are presented as geometric means because the distribution of data generally fit a log-normal distribution, and data were log-normalized to meet normality and constant variance assumptions for analyses of variance (ANOVA). A single ANOVA across years was applied to test the significance of TPAH differences by treatment/reference, however individual ANOVA s were also applied within years to evaluate year-specific conditions.

Overall, mean TPAH increased significantly (by approximately 17x) during and after CTP were removed, in 2014 (48 ng/g wet wt) and 2015 (51 ng/g wet wt) compared to the initial concentration of TPAH in embryos from the high density piling area in 2013 (2.1 to 3.2 ng/g wet wt; ANOVA of log-transformed TPAH by treatment type, $p<0.001$, $F_{62,12}=35.1$, Student-Newman-Keuls multiple range test used for pairwise comparisons – Figure 8, Table 2). In 2014, inshore TPAH levels were statistically indistinguishable from the HDPA samples, and offshore TPAH were lower than inshore and HDPA embryos in 2014. In 2015 this pattern was reversed; TPAH levels in offshore embryos were indistinguishable from the HDPA, while inshore embryos exhibited significantly lower TPAH. Variability in TPAH from all 2015 samples, particularly from inshore and offshore areas, was greater than in 2013 and 2014 (see 95% confidence intervals in Figure 8, Table 2). Mean TPAH in 2013 natural spawn reference samples (0.70 ng/g wet wt) and from CESUs deployed in reference areas (0.56 ng/g wet wt in 2013 and 0.84 ng/g wet wt in 2014) were significantly lower than all treatments.

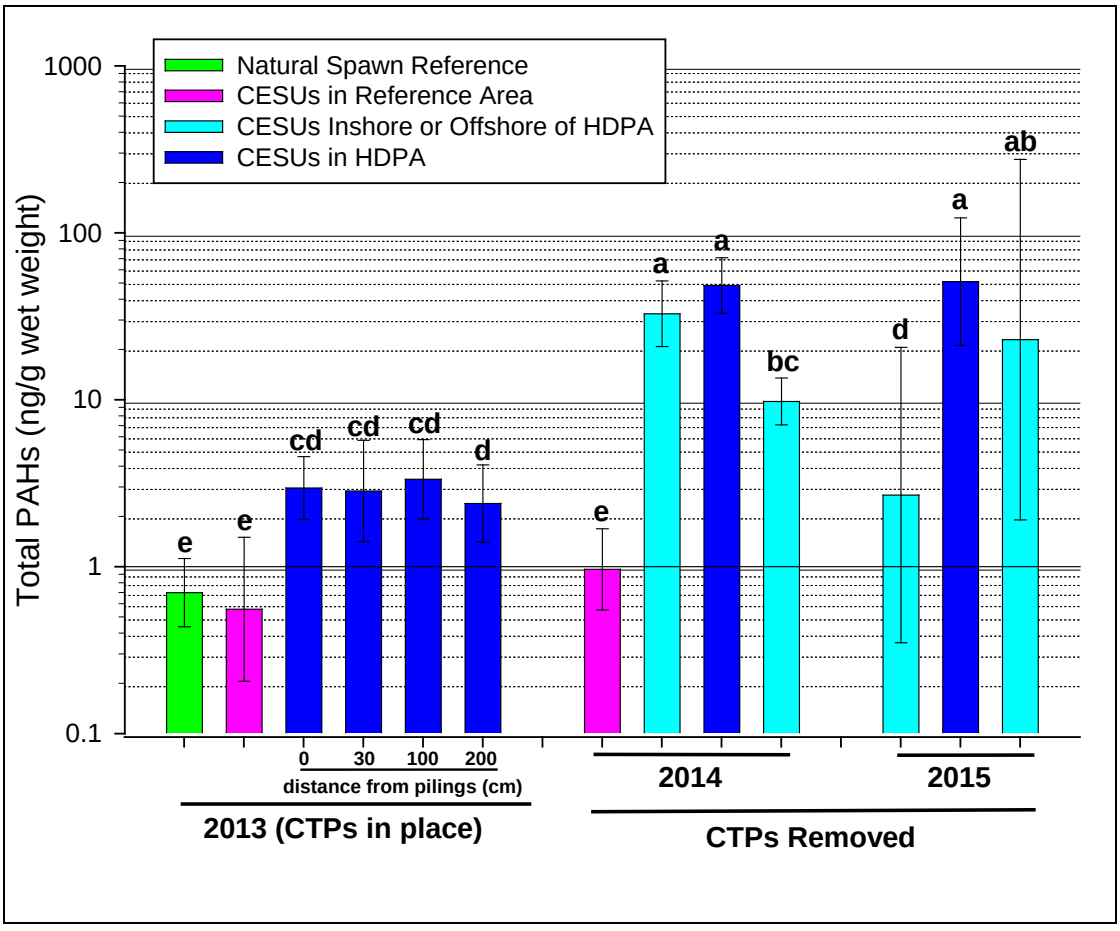


**Figure 8. Mean TPAH (ng/g wet wt, ±95% confidence interval) in herring embryos from treatments and reference areas, for the three yeas of the study. Lower case letters above bars indicate statistical significance of differences between means; different letters indicate significant difference at α=0.05, ANOVA of log-transformed TPAH by treatment; $p<0.001$, $F_{62,12}=35.1$; pairwise comparisons made with a Student-Newman-Keuls multiple range test. Log transformed data met normality assumption for the ANOVA. A slight violation of the homoscedasticity assumption was ignored. Note y-axis is a log10 scale.**

Although TPAH in treatment CESUs deployed at zero to 200 cm from pilings in 2013 were significantly greater than in CESU Reference and Natural Spawn Reference samples in that year, there was no significant difference in TPAH related to distance from pilings within the HDPA (in the range of zero to 200 cm; Figure 8; separate ANOVA of 2013 TPAH by treatment, $F_{19,3}$=0.48, p=0.70). However the power of this comparison was too low (β=0.05) to provide an acceptable chance of observing a true difference, if it existed, between the four treatments within the HDPA.

**Table 2. Summary statistics for total PAH (TPAH) in herring embryos deployed in treatment and reference areas, and in herring embryos spawned naturally on vegetation. Shaded rows indicate TPAH concentration in eggs from ovaries that were used in fertilizations, and in embryos held back (not deployed) during the deployment process.**

| | | | | | Mean | | | Percentile | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Treatment | n | Min | Max | Arith. | Geom. | sd | Median | 10th | 90th |
| 2013 | Natural Spawn | 6 | 0.4 | 1.2 | 0.76 | 0.70 | 0.31 | 0.74 | 0.38 | 1.2 |
| 2013/15 | Deployment Control | 4 | <LOQ | 0.71 | 0.50 | 0.46 | 0.24 | 0.52 | 0.28 | 0.34 |
| 2013 | Reference Area | 3 | 0.4 | 0.7 | 0.58 | 0.56 | 0.20 | 0.69 | 0.35 | 0.71 |
| 2013 | 0cm[a] | 5 | 1.7 | 4.2 | 3.1 | 3.0 | 0.93 | 3.2 | 1.7 | 4.2 |
| 2013 | 30cm | 5 | 1.2 | 4.8 | 3.2 | 2.8 | 1.5 | 3.0 | 1.2 | 4.8 |
| 2013 | 100cm | 5 | 1.8 | 5.2 | 3.6 | 3.3 | 1.4 | 3.1 | 1.8 | 5.2 |
| 2013 | 200cm | 5 | 1.7 | 4.8 | 2.6 | 2.4 | 1.3 | 2.1 | 1.7 | 4.8 |
| 2014 | Maternal Transfer | 3 | 0.63 | 1.7 | 1.2 | 1.1 | 0.59 | 1.2 | 0.66 | 1.6 |
| 2014 | Reference Area | 5 | 0.25 | 1.8 | 0.99 | 0.84 | 0.54 | 0.95 | 0.25 | 1.8 |
| 2014 | Inshore | 4 | 22 | 41 | 34 | 33 | 8.4 | 36 | 22 | 41 |
| 2014 | Former HDPA[b] | 7 | 23 | 87 | 52 | 48 | 20 | 52 | 26 | 81 |
| 2014 | Offshore | 4 | 8.4 | 13 | 9.9 | 9.8 | 2.1 | 9.1 | 8.4 | 13 |
| 2015 | Inshore | 3 | 1.1 | 5.1 | 3.2 | 2.7 | 2.0 | 3.6 | 1.1 | 5.1 |
| 2015 | Former HDPA[b] | 8 | 11 | 180 | 79 | 51 | 71 | 46 | 14 | 180 |
| 2015 | Offshore | 3 | 8.8 | 65 | 32 | 23 | 30 | 21 | 8.8 | 65 |

[a] CESUs deployed zero to 200 cm from pilings in the High Density Piling Area (HDPA)

[b] high density piling area

## PAH Patterns

Forty of 42 quantitated PAH compounds were detected in at least one sample over the three years of this study (Table 3; $C_3$ and $C_4$-Chrysene were never detected). Fifteen PAH compounds (36% of all 42) were detected in 2013, prior to piling removals, and 93% and 86% of compounds were detected in 2014 and 2015, respectively, after pilings were removed.

The relative abundance of individual PAH compounds (expressed as a proportion of TPAH) in embryos from within the piling field (2013) or former piling area (2014 and 2015) appeared similar to the PAH pattern expressed from CTP wood chips sampled during the 2013 CESU deployment (Figure 9). This pattern was characterized by a dominance of 2-ring (acenaphthene), 3-ring (fluorene and phenanthrenes/anthracenes), and 4-ring (fluoranthenes/pyrenes) compounds. Higher molecular weight (5- and 6-ring compounds including benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and

benzo[*e*]pyrene) were rare (<1%) in CTP wood chips and 2013 embryos, but were common (in the range of 1-3%) in 2014 and 2015 embryos. In addition, whereas dibenzothiophenes were present in CTP wood chips and 2014/15 embryos, they were never detected in 2013 embryos. All naphthalene compounds were abundant in CTP wood chips (1-7%), but they were rare in all embryo samples, except for $C_0$ (parent) naphthalene in 2014 embryos. $C_0$ (parent) PAH compounds were always more abundant than their alkylated homologs in cases where the latter were detected.

**Table 3. Frequency of detection (%) of Low Molecular Weight (LMW) and High Molecular Weight (HMW) polycyclic aromatic hydrocarbon compounds (PAHs) in 32 samples of herring eggs collected in 2013, 19 samples in 2014, and 19 in 2015.**

| LMW Compounds | 2013 | 2014 | 2015 | HMW Compounds | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|---|
| Naphthalene | 0% | 29% | 0% | Fluoranthene (FLA) | 69% | 83% | 100% |
| $C_1$-NPH | 0% | 0% | 6% | Pyrene (PYR) | 34% | 83% | 94% |
| $C_2$-NPH | 0% | 4% | 0% | $C_1$-FLA/PYR | 17% | 75% | 88% |
| $C_3$-NPH | 0% | 13% | 29% | $C_2$- FLA/PYR | 0% | 58% | 71% |
| $C_4$-NPH | 0% | 42% | 18% | $C_3$- FLA/PYR | 0% | 38% | 53% |
| Acenaphthylene (ACY) | 0% | 4% | 0% | $C_4$- FLA/PYR | 0% | 8% | 18% |
| Acenaphthene (ACE) | 62% | 79% | 71% | Benz[*a*]anthracene (BAA) | 10% | 54% | 76% |
| Fluorene (FLU) | 66% | 83% | 71% | Chrysene (CHR)[a] | 38% | 67% | 82% |
| $C_1$-FLU | 0% | 54% | 59% | $C_1$-CHR | 0% | 50% | 59% |
| $C_2$-FLU | 0% | 67% | 59% | $C_2$-CHR | 0% | 46% | 41% |
| $C_3$-FLU | 0% | 4% | 12% | $C_3$-CHR | 0% | 0% | 0% |
| Dibenzothiophene (DBT) | 0% | 50% | 59% | $C_4$-CHR | 0% | 0% | 0% |
| $C_1$-DBT | 0% | 25% | 29% | Benzo[*b*]fluoranthene (BBF) | 7% | 50% | 82% |
| $C_2$-DBT | 0% | 33% | 29% | Benzo[*k*]fluoranthene (BKF)[b] | 3% | 50% | 76% |
| $C_3$-DBT | 0% | 42% | 18% | Benzo[*e*]pyrene (BEP) | 0% | 50% | 65% |
| $C_4$-DBT | 0% | 13% | 0% | Benzo[*a*]pyrene (BAP) | 0% | 50% | 65% |
| Phenanthrene (PHN) | 66% | 100% | 65% | Perylene (PER) | 24% | 79% | 71% |
| Anthracene (ANT) | 7% | 58% | 76% | Indeno[*1,2,3-cd*] pyrene (IDP) | 0% | 46% | 53% |
| $C_1$-PHN/ANT | 97% | 100% | 76% | Dibenz[*a,h*]anthracene (DBA)[c] | 0% | 13% | 35% |
| $C_2$-PHN/ANT | 38% | 83% | 88% | Benzo[*z*]pyrene (BZP) | 0% | 46% | 53% |
| $C_3$-PHN/ANT | 3% | 83% | 71% | | | | |
| $C_4$-PHN/ANT | 0% | 13% | 12% | | | | |

[a]coelutes with triphenylene

[b]coelutes with benzo[*j*]fluoranthene

[c]coelutes with dibenz[*a,c*]anthracene

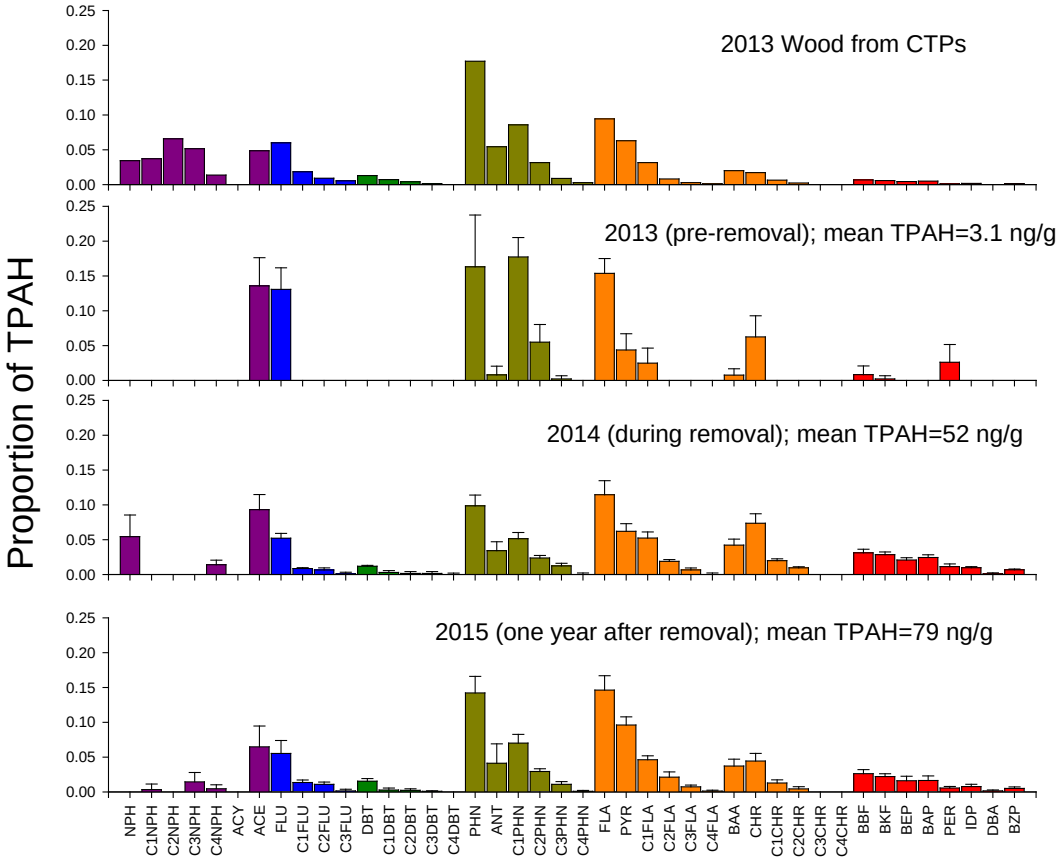


**Figure 9. Relative abundance of individual PAH analytes, as a proportion of total PAHs (TPAH). See Table 3 for full chemical names.**

## Embryo Mortality

Mortality of embryos estimated from three naturally spawned samples in 2013 were low, ranging from 4.3 to 8.0% (Table 4); these samples were taken as an initial estimate of natural background mortality for eggs deposited on natural substrates. Mortality of embryos sampled from CESUs after 10 days of incubation on-site varied widely between 2013 and subsequent years. Mortality was significantly higher for all CESU treatments and reference samples in 2013, ranging from 35-69%, than in 2014 (0-85%, although one 2014 sample exhibited a mortality of 85%, the remainder were less than 40%), and in 2015 (9.2-31%; Table 4; Kruskal Wallis one way ANOVA of ranked Mortality by year, $p<0.001$). Mortalities in 2014 and 2015 were statistically indistinguishable from each other based on this test.

The moderately high mortality of 2013 embryos was consistent across treatments and the reference area; mortality in CESUs deployed in the Fishermen's Point reference area was 35-53%, which was statistically indistinguishable from the treatment CESUs deployed within the high density piling area (43% to 69%; ANOVA of Mortality by sample type, $p=0.36$). This suggested a common source of mortality inherent to the manual spawning technique in 2013, which is discussed below.

**Table 4. Mortality of herring embryos, expressed as a percentage of fertilized eggs that had failed to develop to a 10-day stage after 10 days of incubation.**

| Year | Treatment | n | Minimum | Maximum | Mean | sd |
|---|---|---|---|---|---|---|
| 2013 | Natural Spawn | 3 | 4 | 8 | 7 | 2.0 |
| 2013 | Reference Area | 3 | 35 | 51 | 44 | 8.4 |
| 2013 | 0cm | 5 | 51 | 62 | 54 | 4.6 |
| 2013 | 30cm | 5 | 48 | 59 | 54 | 4.1 |
| 2013 | 100cm | 5 | 43 | 69 | 52 | 10.4 |
| 2013 | 200cm | 5 | 43 | 57 | 51 | 5.7 |
| 2014 | Reference Area | 5 | 0 | 0 | 0 | 0.2 |
| 2014 | Inshore | 4 | 10 | 40 | 24 | 12.3 |
| 2014 | Former HDPA | 7 | 18 | 38 | 28 | 8.6 |
| 2014 | Offshore | 4 | 11 | 85 | 37 | 32.9 |
| 2015 | Inshore | 3 | 9 | 20 | 13 | 6.3 |
| 2015 | Former HDPA | 8 | 9 | 19 | 13 | 3.1 |
| 2015 | Offshore | 3 | 14 | 31 | 21 | 8.7 |

Embryo mortality at the Fishermen's Point reference location was near 100% for all samples deployed there in both 2014 and 2015, for all samples deployed there. The failure of these reference samples in both years is unexplained - CESUs were randomly assigned to these sites in both years, from the common pool of embryos in each year. The high mortality of CESU Reference samples in 2014 and 2015 precluded their use as a reference condition for this study. As an alternative, we included herein five replicate reference samples from the northern shoreline of Hood Head, a reference location approximately 50 km away, in the northern end of Hood Canal. The Hood Head reference samples were collected as part of a concurrent study of PAHs in herring embryos from Port Gamble Bay (West et al. 2015), and were implemented as a clean reference for that study because of the small amount of development along the shoreline, including a lack of CTPs in intertidal or subtidal habitats.

Mortality of embryos increased with increasing TPAH in 2013 and 2014, but not in 2015 (Figure 10; linear regression of mortality by log-transformed TPAH, $p= 0.024$, $p<0.0001$, and $p=0.29$ for 2013, 2014, and 2014, respectively.) The correlation was weak ($r^2=0.22$) in 2013 (Table 5), with TPAH ranging from the limit of quantitation to 5.2 ng/g wet wt; moderately strong ($r^2=0.65$, $p<0.0001$) in 2014, with TPAH ranging from the limit of quantitation to 86 ng/g wet wt; and not significant in 2015 ($p=0.29$).

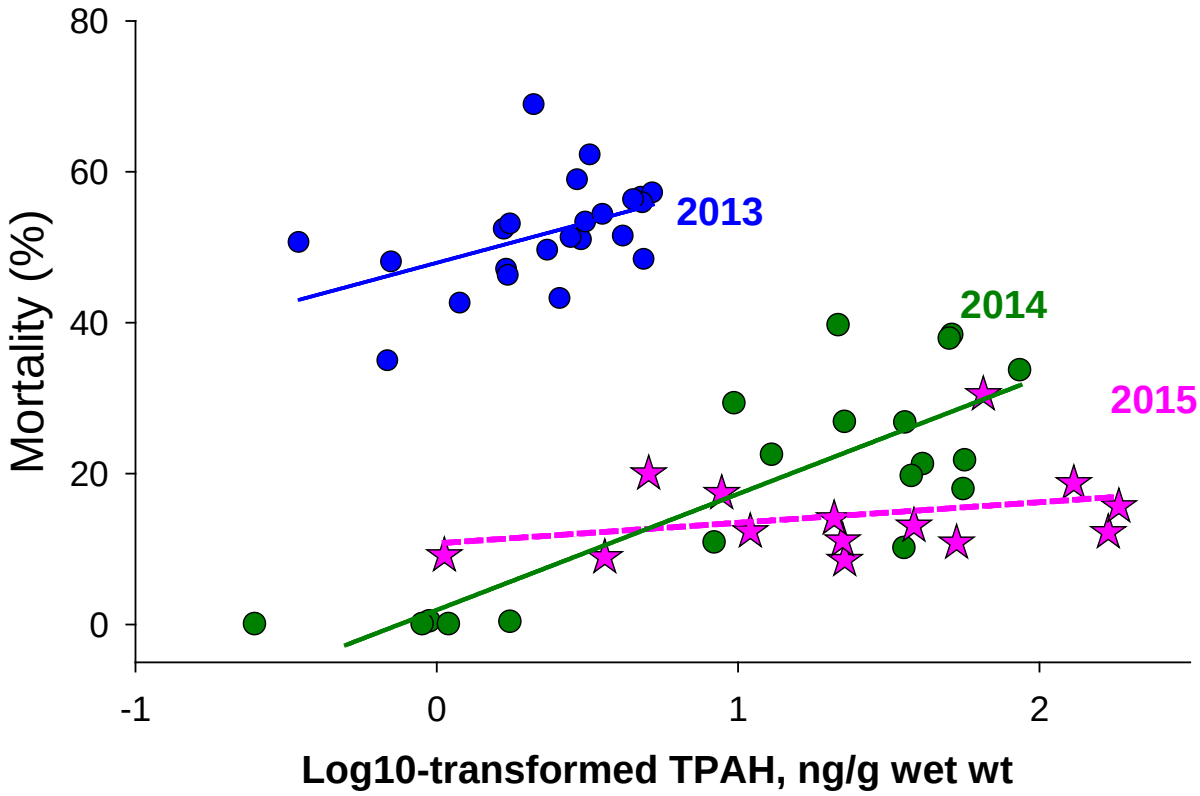


**Figure 10. Mortality of embryos deployed in caged embryo sampling units (CESUs) in reference and treatment areas of Quilcene Bay in 2013, 2014, and 2015. Each symbol represents the mortality estimate (%) and TPAH concentration (ng/g wet wt) for an individual sample. Lines were fitted using least squares linear regression of log10-transformed TPAH data. See Table 5 for regression parameters. A solid line indicates a statistically significant slope; a dashed line indicates insufficient power to detect a trend, if it existed.**

**Table 5. Parameters from linear regressions applied to log10-transformed TPAH and untransformed mortality (%) data for the three years of the study.**

| Parameter | 2013 | 2014 | 2015 |
|---|---|---|---|
| $r^2$ | 0.22 | 0.65 | 0.0189 |
| P | 0.024 | <0.0001 | 0.29 |
| F | 5.89 | 31.7 | 1.25 |
| Sample size | 23 | 19 | 14 |
| Slope | 10.74 | 15.4 | 2.73 |
| Power (β) | 0.62 | 0.99 | 0.17 |
| Intercept | 47.9 | 1.93 | 10.8 |
| Normality Test (p) | 0.3 | 0.95 | 0.016 |
| Constant Variance Test (p) | 0.025 | 0.086 | 0.74 |

## Lipids

Lipid levels were less than 1% in all embryo samples in this study (Table 6). The mean of total extractible lipids of embryos deployed in CESUs ranged from 0.14% in 2013 to 0.46% and 0.54% in 2014 and 2015, and all year-means were significantly different from each other (ANOVA of lipids by year for all CESU embryos, $p<0.001$, $F_{54,2}=187.8$, Student-Newman-Keuls multiple comparison used for pairwise tests.)

**Table 6. Total extractible lipids (%) in embryos deployed in cages at treatment and reference areas, and from naturally spawned embryos**

| | Site Description | N | Mean (%) | minimum | Maximum |
|---|---|---|---|---|---|
| 2013 | Natural Spawn at Reference Area | 6 | 0.08 | <LOQ | 0.02 |
| | Reference Site CESUs (Fish. Pt) | 3 | 0.08 | 0.058 | 0.10 |
| | Treatment - 0 cm | 5 | 0.14 | 0.071 | 0.21 |
| | Treatment - 30 cm | 5 | 0.13 | 0.066 | 0.23 |
| | Treatment - 100 cm | 5 | 0.17 | 0.11 | 0.23 |
| | Treatment - 200 cm | 5 | 0.15 | 0.086 | 0.21 |
| | 2013 Overall | 23 | 0.14 | <LOQ | 0.23 |
| 2014 | Reference Area CESUs (Hood Head) | 5 | 0.48 | 0.45 | 0.52 |
| | High Density Piling Area | 7 | 0.47 | 0.38 | 0.55 |
| | Inshore | 4 | 0.42 | 0.39 | 0.45 |
| | Offshore | 4 | 0.47 | 0.31 | 0.59 |
| | 2014 Overall | 20 | 0.46 | 0.31 | 0.59 |
| 2015 | High Density Piling Area | 8 | 0.56 | 0.44 | 0.71 |
| | Inshore | 3 | 0.44 | 0.39 | 0.51 |
| | Offshore | 3 | 0.57 | 0.53 | 0.60 |
| | 2015 Overall | 14 | 0.54 | 0.39 | 0.71 |

# DISCUSSION

In this study we identified and measured PAH exposure of herring embryos spawned close to creosote-treated pilings (CTP), both before, during, and after CTP removal. The original intent of the study was to measure the effectiveness of removing a potential source of PAH exposure to herring embryos by removing CTPs in a nearshore area where herring normally spawn. However, the original intent of the study was never realized because CTPs were never fully removed from the HDPA piling field. A number of pilings were cut at the seafloor, exposing fresh sources of otherwise largely sequestered creosote, and lengths of cut CTP and CTP fragments were left within the study area.. This resulted in CESUs being placed essentially over newly exposed sources of creosote in the 2014 and 2015 deployments.

WDNR has developed creosote-treated piling removal Best Management Practices (BMP) with the goal to remove as many of the treated pilings as possible with as little impact to Puget Sound as possible. Since 2006 these BMP have been updated based on experience gained in the field managing removal projects. These BMP were used at the Quilcene project site. WDNR found that a much higher percentage of the pilings at this site broke while using the vibratory hammer to try and fully extract the pilings than has occurred with the 50 other piling removal contracts DNR has managed. This unique

circumstance may have occurred due to the conditions of the site such as the age and condition of the pilings and/or the experience level of the contractor removing the pilings.

Underwater surveys of the site following the completion of the contract found approximately 24 pilings in the HDPA that were cut above the mudline, and nine long pilings left on the seafloor. There was also a large amount of creosote-treated woody debris and piling shards left in this location. The DNR contract and BMP require that if the contractor cannot remove the piling with the vibratory hammer that the piling should be cut 1 foot below the mudline and all remnant detached pilings, broken pilings, and debris be removed. The survey found that the piling removal contract was not fully executed per the defined terms, leading to a conclusion that the original intent of the effectiveness monitoring survey could not be fulfilled.

Results for 2013 described the initial condition of TPAH in herring embryos within and near the HDPA, and compared these to caged embryos and naturally spawned embryos from a nearby reference area. The naturally spawned eggs and manually spawned and caged embryos deployed in reference areas in 2013 confirmed that: a) herring naturally spawned along an undeveloped shoreline in the Quilcene Bay area were exposed to extremely low levels of PAHs (i.e., background PAH conditions were low); and b) embryos transplanted to the reference area showed similarly low TPAH concentration, indicating that the deployment procedure successfully mimicked the natural condition. Because the concentration of TPAH in the naturally spawned and reference embryos was similar to conditions in eggs from the ovaries used to create the embryos, the source of the (albeit trivial) PAHs in the natural and reference embryos may have been maternal.

The PAHs observed in embryos in the HDPA in 2013, and the former HDPA area in 2014 and 2015, most likely originated from the CTPs, exposing embryos to high enough concentrations to affect their survival. Highest concentrations of TPAH were observed in the HDPA embryos in all years, which significantly dropped off in the inshore and offshore areas (up to 10 m away), and were at background (near zero) in reference areas (more than 1500 m away). Even though embryos incubating near the derelict CTPs in 2013 (prior to the CTP removal) exhibited relatively low levels of PAHs overall, their mortality was correlated with PAH concentration, and the pattern of PAH analytes in the embryos (in all years) was nearly identical to CTP wood. The TPAH:mortality relationship exhibited high variability, which could have been related to: 1) small-scale variation in PAH exposure based on water-current patterns, 2) variability in individual PAH exposure of embryos related to cage artifacts, 3) variability in susceptibility of individual embryos to PAHs, or 4) inadequate measures of effects. Clearly the sample size was insufficient to adequately model the TPAH:mortality relationship in 2015, if one existed, based on the variability in that year. Although it is likely that all these factors affected the accuracy of the model, it is also likely that embryo mortality to 10 dpf was too coarse a metric of embryo health for this purpose. Virtually all of the most recent studies examining the effects of PAH on fish embryo health have reported sublethal effects, especially including cardiotoxicity (Incardona et al., 2009; Incardona et al., 2004) that would affect survival after hatching. Such metrics were beyond the scope of this study.

The maximum TPAH levels in 2013 Quilcene Bay embryos (5.2 ng/g wet wt) was substantially lower than a TPAH health-effects threshold proposed by Carls et al. [1999; approximately 26 ng/g TPAH wet wt as a

lowest observed effects concentration(LOEC)], suggesting their 26 ng/g LOEC threshold was not protective in the Quilcene Bay case. However the Carls et al. (1999) threshold was based on exposing developing herring embryos to the water-accommodated fraction[3] of weathered crude oil, which presented a substantially different pattern of PAHs, dominated by alkylated homologs of naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes. Moreover, TPAH calculated by Carls et al. (1999) included 31 analytes, which was 11 fewer analytes than the current study. PAHs in the Quilcene Bay embryos were dominated by $C_0$ parent compounds of phenanthrene and fluoranthene, as well as a number of 5- and 6-ring compounds not observed in the Carls study. The mortality patterns observed in this study suggest that creosote-derived PAHs may be toxic to embryos concentrations as low as 3-5 ng/g wet wt.

The pattern of mortality increasing with TPAH concentration in embryos was also observed in 2014, a few weeks after the last CTPs were remove from Quilcene Bay. The TPAH range in embryos was roughly 15 times greater in 2014 compared to 2013, and mortality increased at a greater rate than in 2013, suggesting a greater TPAH effect on developing embryos in 2014. However, baseline mortality between 2013 and 2014 were substantially different, complicating the comparison between years. The relatively low fertilization rate and high mortality of 2013 embryos overall suggests a lower quality (or health) of embryos at the onset of the 2013 experiment. Moreover, lipid levels in 2013 embryos were approximately one-third to one-quarter of the levels in 2014 and 2015 embryos at retrieval, suggesting some difference in metabolism or lipid levels at fertilization, possibly related to egg quality. The unique 2013 embryo conditions may have resulted from an unexpectedly long time between gonad collection and fertilization, which became necessary because poor weather prevented timely deployment of embryos in 2013. Although Dinnell et al. (2011) suggested herring gametes can be stored for five days prior to fertilization and still achieve high fertilization rates (>80%), we observed lower fertilization (>60%) after holding gametes for three days in 2013 (2014 and 2015 eggs were fertilized <24 hrs after collection, and exhibited >95% fertilization). This may further explain the relatively low survival we observed overall for the 2013 embryos. In any case, the fertilization effect was randomly distributed across all treatment and reference samples in 2013, and so should not have confounded the design and results within that year.

The heavy mortality of embryos in CESUs deployed at the Fishermen's Point reference area in 2014 and 2015 remains unexplained. We observed dead embryos that had been naturally spawned on algae (*Sargassum* sp.) adjacent to the retrieved CESUs, so the phenomenon was not solely limited to embryos deployed in cages. The mortality of reference samples in 2014 and 2015 was likely unrelated to the experiment or its design, and it was almost certainly unrelated to PAHs.

The tissue PAH concentration and mortality data combine to strongly suggest that PAHs were released from old, derelict CTPs at levels high enough to affect survival when embryos incubated within 200 cm of the CTPs. The age of the CTPs in the Quilcene Bay railroad trestle was likely at least 90 years; construction probably occurred around 1925 (Christopherson, 2012), suggesting that normal weathering

[3] Chemicals dissolved into water from the previously liquid or solid phase

of CTPs pilings did not necessarily remove PAH risk to fish embryos on a 100-year time scale, and that herring spawning in that area have likely experienced PAH-related mortality for close to a century.

This study also showed that incomplete removal of CTPs and the subsequent exposure of fresh creosote from interior wood surfaces resulted in an increase of PAH exposure to embryos. It is impossible to infer exactly which CTP removal activities were responsible for the increase in PAHs, however the most likely sources included: 1) CTPs cut at the seafloor, leaving the cut surface exposed, resulting in the release of PAHs otherwise sequestered from the interior wood of the CTP, 2) incomplete removal of wood splinters generated from the crushing force of the CTP-grasping machinery (likely related to the brittle nature of the 100-year-old CTPs), which fell to the seafloor, 3) incomplete cleanup of broken piling sections, whether generated from previous processes (e.g., storms) or from the removal procedures, and 4) disturbance of contaminated sediments from pulling pilings out of the substrate. It is unclear how much the unusually brittle nature of the 100-year-old CTPs contributed to the wood debris created from the removal. In any case, this study illustrates the potential hazards associated with releasing creosote-related toxic contaminants from activities associated with incomplete removal of pilings or piling material.

Due to the incomplete removal that occurred at this site, WDNR is reviewing options to complete the cleanup at Quilcene as well as continue to improve the programs BMP[4]. WDNR is developing a plan and methods to clean up the debris and piling stubs that remain at the Quilcene site. Due to the difficulty of removing piling stubs that are 1 foot above the mudline WDNR is examining other methods for removal versus having to cut the pilings again and expose new PAHs. A pilot study will occur during the summer of 2016 to test a new device that may be able to fully extract piling stubs. If this device is successful, full extraction of the piling stubs at Quilcene may occur. Moreover, WDNR will improve underwater enforcement of BMPs by completing an independent underwater survey to review work before a contract is complete. This will help ensure that the contractor has cut pilings according to the BMP as well as removed any creosote-treated debris that is on the seafloor.

[4] Personal communication, Monica Shoemaker, Washington Department of Natural Resources; monica.shoemaker@dnr.wa.gov

# ACKNOWLEDGEMENTS

All chemical analyses in this study were performed by the Environmental Chemistry Division at NOAA's Northwest Fisheries Science Center under the guidance of Gina Ylitalo.

This intensive field and lab study was not possible without the help of a number of key contributors:

Point Whitney/Port Townsend WDFW Field Office crew for their help and use of lab space (Rich Childers, Doug Rogers, Camille Speck, and Paul Clarke)

The Troutlodge Facility, Brinnon, WA (John Dentler, Megan Sorby, and Tom Sorby)

WDFW's Subtidal Shellfish Dive Unit (Bob Sizemore, Ocean Eveningsong, Jessi Shultz, Bethany Stevick, Michael Ulrich, and Lisa Hillier)

WDFW's Oil Spill Response team for their help collecting adult herring, deploying & retrieving cages and for the use of their Boston Whaler and Almar (Don Noviello, Brian MacDonald, and Dan Doty)

NOAAs Northwest Fisheries Science Center staff John Incardona, Tiffany Linbo, and Richard Edmunds)

NewFields/Port Gamble Environmental Sciences Laboratory/Environ (Brian Hester, Collin Ray, Jay Word, and Meg Pinza)

WDFW's Forage Fish Unit for the use of the *Malolo* and for diving on cages and recording video (Taylor Frierson and Larry Leclair)

Other WDFW staff (Stephanie Karney and Kwasi Addae)

Volunteers (Mary Ann Wie)

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# ABBREVIATIONS

AH – aromatic hydrocarbon

ASE accelerated solvent extraction

CESU- caged embryo sampling unit

CTP- creosote-treated piling

GC/MS - gas chromatography/mass spectrometry

HDPA- high density piling area

PAH- polycyclic aromatic hydrocarbon

SEC HPLC - size-exclusion high-performance liquid chromatography

SIM - selected-ion monitoring

wt - weight

# SUPPLEMENTAL INFORMATION

**Supplemental Table 1. Collection location and capture information for adult Pacific herring over the three years of the study.**

| | Set # | Latitude | Longitude | Net Size (ft) and type | Soak Time | Total Herring Collected |
|---|---|---|---|---|---|---|
| 2013 | 2 | 47.77940 | -122.84218 | 100 x 12[a] | 50 min | 8 |
| | 3 | 47.78018 | -122.85310 | 100 x 12[a] | 4 hr 33 min | 5 |
| | 4 | 47.77940 | -122.84218 | 100 x 12[a] | 3 hr 30 min | 46 |
| | 5 | 47.78102 | -122.83587 | 200 x 12[a] | 3 hr 20 min | 1 |
| | 6 | 47.77940 | -122.84218 | 100 x 12[a] | 35 min | 51 |
| | 7 | 47.78273 | -122.83308 | 200 x 12[a] | 20 min | 137 |
| | 8 | 47.78018 | -122.85310 | 100 x 12[a] | 1 hr 15 min | approx. 210 |
| 2014 | 01A | 47.78083 | -122.83769 | 100 x 11 sinking[b] | 27 min | 1 |
| | 01C | 47.78788 | -122.82360 | 100 x 12 floating[b] | 34 min | 1 |
| | 02A | 47.78056 | -122.83757 | 100 x 11 sinking[b] | 1 hr 48 min | 2 |
| | 04A | 47.77900 | -122.85129 | 100 x 11 sinking[b] | 1 hr 30 min | 65 |
| 2015 | 1 | 47.78029 | -122.83933 | 200 x 12 floating[a] | 3 hr 10 min | unrecorded |
| | 5 | 47.77935 | -122.84744 | 100 x 11 sinking[b] | 3 hr 30 min | 200 |

[a] 1.75 inch mesh size

[b] 1.5 inch mesh size

**Supplemental Table 2. Location of caged embryo sampling units (CESU) deployed in the three years of the study.**

| | Site Description | CESU # | Latitude | Longitude |
|---|---|---|---|---|
| 2013 | Fishermen's Point | 46 | NA | NA |
| (CESU n = 23) | Reference Site | 65 | NA | NA |
| | | 80 | NA | NA |
| | Inside the High | 177, 296, 380, 92 (1) | 47.79781845 | -122.867873 |
| | Density Piling Area | 369, 245, 280, 39 (64) | 47.79777572 | -122.867888 |
| | | 238, 254, 431, 73 (171) | 47.79789895 | -122.8678601 |
| | | 316, 411, 251, 37 (261) | 47.79785986 | -122.8678611 |
| | | 403, 165, 161, 55 (389) | 47.79786652 | -122.8679413 |
| 2014 | Fishermen's Point, | 330 | 47.77975350 | -122.85292110 |
| (CESU n = 19) | Reference Site | 331 | 47.77963820 | -122.85091470 |
| | | 327 | 47.77962[a] | -122.85061[a] |
| | | 328 | 47.77951[a] | -122.84985[a] |
| | Former High | 329 | 47.79798830 | -122.86773110 |
| | Density Piling Area | 321 | 47.79782460 | -122.86804180 |
| | | 325 | 47.79789590[b] | -122.86782100[b] |
| | | 322 | 47.79789590[b] | -122.86782100[b] |
| | | 323 | 47.79788510 | -122.86793400 |
| | | 324 | 47.79801370 | -122.86785400 |
| | | 308 | 47.79784100 | -122.86780680 |
| | Inshore | 304 | 47.79829570 | -122.86780850 |
| | | 303 | 47.79818330 | -122.86771960 |
| | | 305 | 47.79797500 | -122.86814420 |
| | | 302 | 47.79768820 | -122.86809040 |
| | Offshore | 316 | 47.79811210 | -122.86759610 |
| | | 314 | 47.79791470 | -122.86756110 |
| | | 319 | 47.79776100 | -122.86767000 |
| | | 320 | 47.79766080 | -122.86769130 |
| 2015 | Fishermen's Point, | 129 | 47.77955 | -122.8497 |
| (CESU n = 17) | Reference Site | 130 | 47.77892 | -122.8426 |
| | | 143 | 47.78091 | -122.837 |
| | Former High | 181 | 47.79788 | -122.8679 |
| | Density Piling Area | 182 | 47.79781 | -122.8678 |
| | | 183 | 47.79794 | -122.8678 |
| | | 184 | 47.79777 | -122.8679 |
| | | 185 | 47.79771 | -122.8679 |
| | | 186 | 47.79776 | -122.868 |
| | | 187 | 47.79784 | -122.868 |
| | | 188 | 47.79760 | -122.8679 |
| | Inshore | 160 | 47.79787 | -122.8682 |
| | | 163 | 47.79778 | -122.8682 |
| | | 164 | 47.79766 | -122.8682 |
| | Offshore | 189 | 47.79790 | -122.8677 |
| | | 190 | 47.79780 | -122.8676 |
| | | 191 | 47.79760 | -122.8676 |

[a] CESU coordinates inadvertently not recorded on the day of retrieval. These are coordinates recorded on the day of deployment using a less accurate GPS

[b] the lines of the buoys from CESUs 325 and 322 were twisted together upon retrieval resulting in identical site coordinates. The actual location for these CESUs is less accurate than the other CESUs, but likely close to those presented here

# CASE NARRATIVES

The following case narratives detail quality assurance and quality control measures related to the batches of herring embryo samples that were analyzed for PAHs and lipids.

## 2013 PSEMP Herring study: analyses of embryos for polycyclic aromatic hydrocarbons (PAHs)

Analyses of herring embryos for polycyclic aromatic hydrocarbons (PAHs) by gas chromatography/mass spectrometry (GC/MS)

Sets PS2948, PS2949, PS2950

*Surrogate Recoveries*
Surrogate recoveries for herring embryo samples and all quality assurance samples method blanks and NIST Standard Reference Material (SRM) 1974c] associated with the analyses of these samples were within the guidelines detailed in the QAP (recoveries are to be between 60-130%).

*Sample Replicates*
One herring embryo sample (13QB-PHSE32A in set PS2950) was analyzed in triplicate and the criteria in the QAP were met for all analytes (RSDs are to be ≤ 15% for ≥ 90% of the analytes that have concentrations ≥ 1 ng/g).

*Method Blank Analysis*
A method blank was analyzed for PAHs with each sample set. The criteria in our Quality Assurance Plan (QAP) (Sloan et al. 2006) for method blanks were met [no more than 5 analytes in a method blank, excluding alkyl-substituted PAH homologue groups, are to exceed 2 × the lower limit of quantitation (LOQ)]. However, it should be noted that these samples were analyzed with a lower lowest level calibration standard than applicable in the QAP criteria.

*Standard Reference Material (SRM) Analyses*
An aliquot of NIST SRM 1974c (blue mussel) was analyzed with each of these sample sets. The results met the criteria in the QAP (concentrations of ≥ 70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the NIST SRM 1974c certified values) for set PS2950, but not for PS2948 (60% met) and PS2949 (67% met). 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 uncertified coeluting compounds.

We have noted inadequate performance of one of the C2-naphthalenes [2,6-dimethylnaphthalene (DMN)] as demonstrated by measuring variable concentration of this analyte that are 18 to 43 times higher than the NIST reference value indicating a high bias. As a result of the high bias for this analyte, we recommend that for each field sample, the concentration of DMN reported in the sample be subtracted from the C2-naphthalenes will also affect the reported values for summed low molecular weight PAHs and summed PAHs.

*Calibrations*
Continuing calibration verification standards were analyzed at the start, middle and end of the GC/MS analytical sequence for this set of samples, and all of the results met the continuing calibration criteria detailed in the "Quality Assurance Plan for Analyses of Environmental Samples for Polycyclic Aromatic Compounds, Persistent Organic Pollutants, Fatty Acids, Stable Isotope Ratios, Lipid Classes, and Metabolites of Polycyclic Aromatic Compounds" by Sloan et al., 2006. The relative standard deviation (RSD) of each of the analyte responses relative to the surrogate standard was ≤ 15% for all analytes in these sample sets.

## 2014 PSEMP Herring study: analyses of embryos and ovaries for polycyclic aromatic hydrocarbons (PAHs)

Analyses of herring embryos and ovaries for polycyclic aromatic hydrocarbons (PAHs) by gas chromatography/mass spectrometry (GC/MS)

Sets PS3050, PS3051, PS3054, PS3060, PS3064, PS3065, PS3067

*Surrogate Recoveries*
Surrogate recoveries for herring embryo and ovary samples and all quality assurance samples [method blanks and NIST Standard Reference Material (SRM) 1974c] associated with the analyses of these samples were within the guidelines detailed in the QAP (recoveries are to be between 60-130%), with the exception of dBaP (133%) in sample 119-2287 and SRM1974c 119-2291 (135%), which slightly exceeded the guidelines. However, no analytes calculated versus this surrogate were detected above the LOQ in this sample.

*Sample Replicates*
Two herring ovary samples (14PGB-PHOV03 in set PS3050 and 14PGB-PHOV02 in set PS3060) were analyzed in triplicate and the criteria in the QAP were met for all analytes (RSDs are to be ≤ 15% for ≥ 90% of the analytes that have concentrations ≥ 1 ng/g). In some instances, the concentrations of analytes were so low that they were detected in one sample or two samples but were below the LOQ in the other sample(s) — in these cases the RSD may be >50%, but this is an artifact of the LOQ.

*Method Blank Analysis*
A method blank was analyzed for PAHs with each sample set. The criteria in our Quality Assurance Plan (QAP) (Sloan et al. 2006) for method blanks were met [no more than 5 analytes in a method blank, excluding alkyl-substituted PAH homologue groups, are to exceed 2 × the lower limit of quantitation (LOQ)] with the exception of PS3051, which had 7 analytes exceed 2 × the LOQ. However, it should be noted that these samples were analyzed with a lower lowest level calibration standard than applicable in the QAP criteria.

*Standard Reference Material (SRM) Analyses*
An aliquot of NIST SRM 1974c (blue mussel) was analyzed with each of these sample sets. The results met the criteria in the QAP (concentrations of ≥ 70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the NIST SRM 1974c certified values) for sets PS3050, PS3054, PS3065 and PS3067, but not for PS3051, PS3060, and PS3064 (67% met). 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 uncertified coeluting compounds.

We have noted inadequate performance of one of the C2-naphthalenes [2,6-dimethylnaphthalene (DMN)] as demonstrated by measuring variable concentration of this analyte that are 18 to 43 times higher than the NIST reference value indicating a high bias. As a result of the high bias for this analyte, we recommend that for each field sample, the concentration of DMN reported in the sample be subtracted from the concentration reported for C2-NPH. This recommended change in concentrations of C2-naphthalenes will also affect the reported values for summed low molecular weight PAHs and summed PAHs.

*Calibrations*
Continuing calibration verification standards were analyzed at the start, middle and end of the GC/MS analytical sequence for this set of samples, and all of the results met the continuing calibration criteria detailed in the "Quality Assurance Plan for Analyses of Environmental Samples for Polycyclic Aromatic Compounds, Persistent Organic Pollutants, Fatty Acids, Stable Isotope Ratios, Lipid Classes, and Metabolites of Polycyclic Aromatic Compounds" by Sloan et al., 2006. The relative standard deviation (RSD) of each of the analyte responses relative to the surrogate standard was ≤ 15% for all analytes in these sample sets.

## 2015 PSEMP Quilcene Bay Herring study: analyses of embryos for polycyclic aromatic hydrocarbons (PAHs)

Analyses of embryos (eggs) of herring for polycyclic aromatic hydrocarbons (PAHs) by gas chromatography/mass spectrometry (GC/MS)

Sets PS3202, PS3203

*Calibrations*
Continuing calibration verification standards were analyzed at the start, middle and end of the GC/MS analytical sequence for these sets of samples, and all of the results met the continuing calibration criteria detailed in the "Quality Assurance Plan for Analyses of Environmental Samples for Polycyclic Aromatic Compounds, Persistent Organic Pollutants, Fatty Acids, Stable Isotope Ratios, Lipid Classes, and Metabolites of Polycyclic Aromatic Compounds" by Sloan et al., 2006. The relative standard deviation (RSD) of each of the analyte responses relative to the surrogate standard was ≤ 15% for all analytes in these sample sets.

*Method Blank Analysis*
A method blank was analyzed for PAHs with each sample set. Note that the criteria in our Quality Assurance Plan (QAP) (Sloan et al. 2006) for method blanks [no more than 5 analytes in a method blank are to exceed 2 × the lower limit of quantitation (LOQ)] were written to apply to sets calculated using a specific calibration range; the current sets used an extended calibration range that included a standard with a lower concentration. Therefore, the LOQs reported for these sample sets are ~3 times lower than the LOQs normally reported, and the QAP criteria do not apply. For the current sets, 5 or fewer analytes exceeded 2 × these lower LOQs.

*Surrogate Recoveries*
Surrogate recoveries for herring egg samples and all quality assurance samples [method blanks and NIST Standard Reference Material (SRM) 1974c] associated with the analyses of these samples were within the guidelines detailed in the QAP (recoveries are to be between 60-130%).

*Sample Replicates*
No replicates were analyzed with these sample sets

*Standard Reference Material (SRM) Analyses*
An aliquot of NIST SRM 1974c (blue mussel) was analyzed with each of these sample sets. The results met the criteria in the QAP (concentrations of ≥ 70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the NIST SRM 1974c certified values) for all sets. 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 uncertified coeluting compounds.

We have noted inadequate performance of one of the C2-naphthalenes [2,6-dimethylnaphthalene (DMN)] as demonstrated by measuring variable concentration of this analyte that are 18 to 43 times higher than the NIST reference value indicating a high bias. As a result of the high bias for this analyte, we recommend that for each field sample, the concentration of DMN reported in the sample be subtracted from the concentration reported for C2-NPH. This recommended change in concentrations of C2-naphthalenes will also affect the reported values for summed low molecular weight PAHs and summed PAHs.