

Pacific Herring Studies

The Washington State Department of Ecology (Ecology) is funding two Pacific herring studies in Port Gamble Bay – an embryo mortality study and a genetic study. These studies will be conducted by the Washington State Department of Fish and Wildlife (WDFW) with assistance from the Port Gamble S’Klallam Tribe for the embryo work.

The embryo mortality study will build on previous studies and ongoing work by WDFW to provide baseline information on herring embryo contamination and health that can be used to evaluate the effectiveness of the cleanup remedy in the future. In addition this work may provide insight into the trend of decreased herring spawning in the bay. The genetic study will obtain genetics samples from several herring populations in order to better identify relationships among spawning stocks and provide a baseline condition that can be used to evaluate future interactions among stocks.

Prior to 2000 the Port Gamble Bay herring stock was considered one of the larger spawning stocks in Puget Sound (seventh largest based on spawning biomass estimates from 2003 to 2012). Spawning biomass has steadily declined from 2,459 tons in 2003 to 273 tons in 2012 (WDFW in preparation). Pacific herring, the cornerstone of the marine food web, are a critically important food source for salmon and other fish, marine mammals, and marine birds. Given their importance, this decline is unexplained, is cause for concern, and provides motivation for the current studies.

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Photo Credit to Washington State Department of Fish and Wildlife

Detailed Project Descriptions

Embryo Mortality Study

Unusually high rates of embryo mortality (>20%) have been observed in Port Gamble Bay since the early 1980s (WDFW unpublished data). Since that time efforts have been made to assess embryo mortality and its possible link to contamination (Kocan 1987). These efforts have compared polycyclic aromatic hydrocarbons (PAH) concentrations and embryo health with other spawning stocks (Hershberger et. al. 2005), and used blue mussels (*Mytilus galloprovincialis*) as a proxy for PAH exposure in herring embryos (Applied Biomonitoring 2002). These studies, as well as a recent assessment of PAH concentrations in herring embryos from five spawning stocks in Puget Sound (not including Port Gamble Bay; West et al. in review), have implicated PAHs as a risk to herring embryo health in Puget Sound. Moreover, lab and field studies have demonstrated clear links between exposure of herring embryos to aqueous PAH

concentrations and sublethal effects, such as cardiac edema and arrhythmia (Incardona et al. 2009, Incardona et al. 2004).

PAH exposure of embryos from creosote-treated pilings has also been identified as a potential source of mortality in developing herring embryos. Vines et al. (2000) demonstrated toxicity of diffusible creosote-derived compounds on herring embryos using a variety of health effects endpoints including cardiac function, embryo movement, hatching success and larval morphology. Toxicological effects of PAHs on herring embryo health can also be exacerbated with addition of ultraviolet light (e.g., from sunlight; Barron et al. 2003, Barron et al. 2005, Hatlen et al. 2010).

These studies, combined with the PAH conditions in Port Gamble Bay sediments identified by Ecology (2012) and Long (et al. 2003), form a weight of evidence that elevates suspicion of PAH contamination as a cause of herring embryo mortality in Port Gamble Bay.

The purpose of the current study is to evaluate the spatial extent of PAH contamination in herring embryos spawned in Port Gamble Bay, and compare contamination levels with measures of embryo health. This investigation will attempt to answer the following key questions:

1. Is PAH contamination occurring in Port Gamble Bay?
2. Where is the contamination occurring?
3. What is the magnitude of contamination?
4. What are the health effects for herring embryos exposed to PAHs?

This study will build on WDFW's previous Puget Sound Ecosystem Monitoring Program (PSEMP) work and will link with ongoing annual herring spawn distribution surveys conducted by WDFW's Forage Fish Unit.

Methodology

WDFW will develop a study design in a Quality Assurance Project Plan (QAPP) prior to initiation of field work. Because herring typically do not use all available habitat in any given year and extent of spawning habitat use is unpredictable, this study will rely on placing herring embryos derived from manually spawned eggs in a pre-determined pattern across Port Gamble Bay and reference/control areas.

Briefly, this involves:

- collecting spawn-ready adults during the peak of the spawning season,
- extracting gametes from adult fish and manually placing the eggs onto mesh panels where they will be fertilized (Figure 1),
- placing mesh panels into anti-predator cages deployed at pre-determined locations,
- allowing embryos to incubate *in situ* for up to ten days,
- recovering caged embryos,
- sampling embryos from the mesh panels for chemical analyses, and
- evaluating embryos for health effects endpoints.



Figure 1. Egg fertilization and cage construction in the laboratory. Clockwise from left: replicate Nitex mesh sheets with monolayers of eggs incubating in milk; insertion of Nitex sheet with fertilized eggs into cage; fully assembled cage, or CESU. Photo courtesy of John Incardona, NOAA Fisheries.

This technique parallels work being conducted by WDFW/PSEMP in a Quilcene Bay study evaluating the effects of diffusible creosote-derived compounds on herring embryos (West 2013).

Approximately 38 PAH compounds or groups will be analyzed in this study and include the 18 low molecular weight (LMW) and 20 high molecular weight (HMW) analytes identified below. Alkylated homolog groups are indented.

Low Molecular Weight	High Molecular Weight
acenaphthylene (ACY)	fluoranthene (FLA)
acenaphthene (ACE)	pyrene (PYR)
fluorene (FLU)	C ₁ -fluoranthenes/pyrenes
C ₁ -fluorene	C ₂ -fluoranthenes/pyrenes
C ₂ -fluorenes	C ₃ -fluoranthenes/pyrenes
C ₃ -fluorenes	C ₄ -fluoranthenes/pyrenes
dibenzothiophene (DBT)	benzo[<i>a</i>]anthracene (BAA)
C ₁ -dibenzothiophene	chrysene (CHR) ^b
C ₂ -dibenzothiophenes	C ₁ -chrysene
C ₃ -dibenzothiophenes	C ₂ -chrysenes
C ₄ -dibenzothiophenes	C ₃ -chrysenes
phenanthrene (PHN)	C ₄ -chrysenes
anthracene (ANT)	benzo[<i>b</i>]fluoranthene (BBF)
C ₁ -phenanthrenes/anthracenes	benzo[<i>k</i>]fluoranthene (BKF) ^c
C ₂ -phenanthrenes/anthracenes	benzo[<i>e</i>]pyrene (BEP)
C ₃ -phenanthrenes/anthracenes	benzo[<i>a</i>]pyrene (BAP)
C ₄ -phenanthrenes/anthracenes	perylene (PER)
retene ^a	indeno[1,2,3- <i>cd</i>]pyrene (IDP)
	dibenz[<i>a,h</i>]anthracene (DBA) ^d
	benzo[<i>z</i>]pyrene (BZP)

^a1-methyl-7-isopropyl phenanthrene, individual analyte, included in C₄-P/A

^bcoelutes with triphenylene

^ccoelutes with benzo[*j*]fluoranthene

^dcoelutes with dibenz[*a,c*]anthracene

Schedule

The embryo mortality work will be completed in conjunction with WDFW's annual herring spawn deposition surveys, and during peak spawning of the Port Gamble stock. Field work will be completed in the winter of 2014. Chemical analysis will take up to 6 months to complete. Following receipt of the lab results, WDFW will analyze and summarize the findings in a written report.

Genetic Study

Previous genetic studies designed to evaluate population connectivity among Puget Sound herring stocks have produced mixed results for Port Gamble herring, suggesting that reproductive isolation may be occurring but may not be stable across years (Small et al. 2005). Further evaluation of stock genetics using next-generation genetic tools, which requires fewer samples to make valid comparisons, is warranted for use in fishery and ecosystem management.

The current study is intended to leverage annual herring spawn distribution surveys conducted by WDFW, supplemented by targeted gillnetting, to obtain genetics samples from several herring populations in order to elucidate relationships among spawning stocks. This study will provide a baseline condition that can be used to evaluate future interactions among stocks following cleanup efforts and/or Sound-wide environmental perturbations. This investigation will attempt to answer the following key questions:

1. Is the Port Gamble herring stock genetically distinct, and reproductively isolated from other nearby herring stocks?
2. Is the Port Gamble herring stock genetically distinct, and reproductively isolated from Puget Sound herring stocks at large?
3. Is the Port Gamble herring stock genetically distinct, and reproductively isolated from other Puget Sound herring stocks at a level that warrants special management consideration, as with the Cherry Point and Squaxin Island stocks?

The WDFW conducts spawning ground surveys for all known herring stocks in Puget Sound on an annual basis during stock-specific spawn timing windows. Though eggs collected during these surveys have been used in previous and ongoing genetic studies, the potential for collection of siblings, which biases the results when small sample sizes are used, is a significant concern. To avoid this concern, fin clips from adult herring will be used in this study.

Methodology

When adult herring are abundant on the spawning grounds, WDFW biologists will use gill nets fished from a research vessel to obtain a sample of adults spanning the size and age range of adult fish in the aggregation. Gillnetting will occur over the course of 2 to 3 days per stock per season, or until 75 fish are collected. Samples will be taken over the course of two sampling seasons with a target of 75 fish per population per year for six spawning stocks. The spawning stocks selected for this study constitute a range of spatially proximate, genetically distant, and “reference” populations that will allow direct evaluation of the three key questions laid out above. These stocks include the Port Gamble, Quilcene, Holmes Harbor, Cherry Point, Port Orchard/Port Madison, and Squaxin Island stocks.

After collection, fin clips will be taken from individuals and stored in 95% non-denatured ethanol. Samples will then be transferred to the WDFW genetics laboratory for analysis. Depending on available reference libraries and processing tools, the laboratory will then use either microsatellites or single nucleotide polymorphisms (SNPs) to quantitatively evaluate relationships among herring populations.

Schedule

Genetic sampling will be completed concurrent with the WDFW’s annual herring surveys and across the breadth of the spawning season in 2014 and 2015. For most stocks this period ranges from January through April of each year, but for the Cherry Point stock this period is from March through June.

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