Puget Sound Steelhead Marine Survival: Draft 2017-2019 Research Work Plan

Puget Sound Steelhead Marine Survival Workgroup

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

Steelhead trout are the official fish of Washington State, an icon of the Pacific Northwest, and a major contributor to Washington's recreation and fishing economies. Yet the Puget Sound steelhead population, listed as threatened under the Endangered Species Act in 2007, is now less than 10% of its historic size and faces possible extinction. Poor juvenile survival in the Puget Sound marine environment has been identified as key factor in that decline and a significant barrier to recovery.

Millions of dollars have been spent over the past decade to recover wild steelhead in Puget Sound. Finding a solution to high marine mortality rates of juvenile fish would protect that investment and boost economic activity in communities around the Sound that benefit from viable steelhead fisheries.

In 2013, the Washington Department of Fish and Wildlife and the Puget Sound Partnership initiated an effort to determine why juvenile steelhead are dying in Puget Sound.[1] This collaborative effort involves state and federal agencies, Puget Sound Treaty Tribes, nonprofits, and academia. It is coordinated by the nonprofit, Long Live the Kings, and is a component of the Salish Sea Marine Survival Project. The work has been funded by $1.6 million in Washington State appropriations and over $1.0 million in direct match in equipment, services, staff time, and other funding from collaborators.

Through thirteen studies implemented to date, the Puget Sound Steelhead Marine Survival Workgroup determined that the causes of mortality are most likely derived in the lower-river or marine environments and predation and disease are likely the most significant factors affecting survival. In some Puget Sound estuaries, the parasite *Nanophyetus salmincola* is present at high levels and may reduce swimming performance or directly cause mortality. Contaminants in the Nisqually River also negatively impact steelhead health. Compromised fish may be more susceptible to predation, which is likely the immediate cause of most juvenile steelhead mortality within Puget Sound. Harbor seal populations in Puget Sound have nearly tripled since the 1980s, and scat and acoustic telemetry analyses indicate seal predation on juvenile steelhead. Other potential steelhead predators include harbor porpoises and cormorants. In 2016 and 2017, the early marine survival of Nisqually steelhead more than doubled. Initial information suggests that significant changes in the Puget Sound marine environment may have reduced steelhead predation risk (e.g. anchovy in high abundance and the presence of transient killer whales). This new information is contributing to our understanding of predation dynamics and factors that may mitigate or exacerbate predation on steelhead populations. Please see Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2017 research findings summary[2] for more information.

A third and final Washington State appropriation of $750,000 was provided in the 2017-2019 supplemental budget round in early 2018. In this final phase of research of steelhead early marine survival research, the Workgroup will:

[1] Steelhead Marine Survival Workgroup. February 2014. Salish Sea Marine Survival Project - Research Work Plan: Marine Survival of Puget Sound Steelhead. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com

[2] Puget Sound Steelhead Marine Survival Workgroup. 2018. Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2017 research findings summary. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com.

1) Continue to assess steelhead early marine survival rates, predation, and factors that may affect the extent of predation including hatchery release magnitude and timing, forage fish abundance, and presence/absence of transient whales.
2) Complete an assessment of ecosystem dynamics that have changed over the past 30 years relative to the trends in steelhead marine survival.
3) Re-examine the extent to which the *N. salmincola* parasite leads directly or indirectly to mortality.
4) Identify *N. salmincola* hotspots in the Nisqually River and recommend actions to reduce their loads.
5) Complete the work to isolate the sources of contaminants in the Nisqually River.

The Puget Sound Steelhead Marine Survival Workgroup is also providing the Puget Sound Steelhead Recovery Team a report regarding early marine mortality constraints and management options to test, to inform the final Puget Sound Steelhead Recovery Plan due out in late 2018.

2017-2019 RESEARCH

From January through September 2017, the Workgroup reviewed their findings to date and developed and prioritized studies based upon those findings. This section describes the framework and proposed studies for the 2017-2019 research phase.

Framework

A three-question research framework was established by the Workgroup during their initial round of research (2013-2015). The questions are:

Q1. What is the survival history of Puget Sound steelhead and where, when and at what rate is mortality occurring now? How do the abundance and marine survival trends of Puget Sound steelhead populations (hatchery and wild) compare to other Pacific Coast populations, especially other regions of Washington State (e.g., lower Columbia and coast) and the Strait of Georgia? How do the abundance trends, marine survival trends, and early marine mortality rates and locations of mortality vary among populations within Puget Sound?

Q2. What is the direct/proximate[3] cause of mortality in Puget Sound?

Q3. What is leading to this mortality? What are the root/underlying causes? Are they freshwater and/or marine derived?

The Workgroup continues to use this research framework, and the associated logic model diagram, below (**Error! Reference source not found.**), to categorize their assumptions and supporting evidence. The assumptions based upon research to date are summarized in the diagram below. The evidence supporting the assumptions is described in the Research Work Plan: Marine Survival of Puget Sound Steelhead (2014)[4], and the 2013-2017 findings summary[5]. This information provides the basis for the Workgroup's affiliated research for 2017-2019 work phase.

[3] The Workgroup defines direct or proximate causes of mortality as those that result in the immediate death of juvenile steelhead.

[4] Puget Sound Steelhead Marine Survival Workgroup. February 2014. Salish Sea Marine Survival Project - Research Work Plan: Marine Survival of Puget Sound Steelhead. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com.

[5] Puget Sound Steelhead Marine Survival Workgroup. September 2018. Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2017 research findings summary. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com.

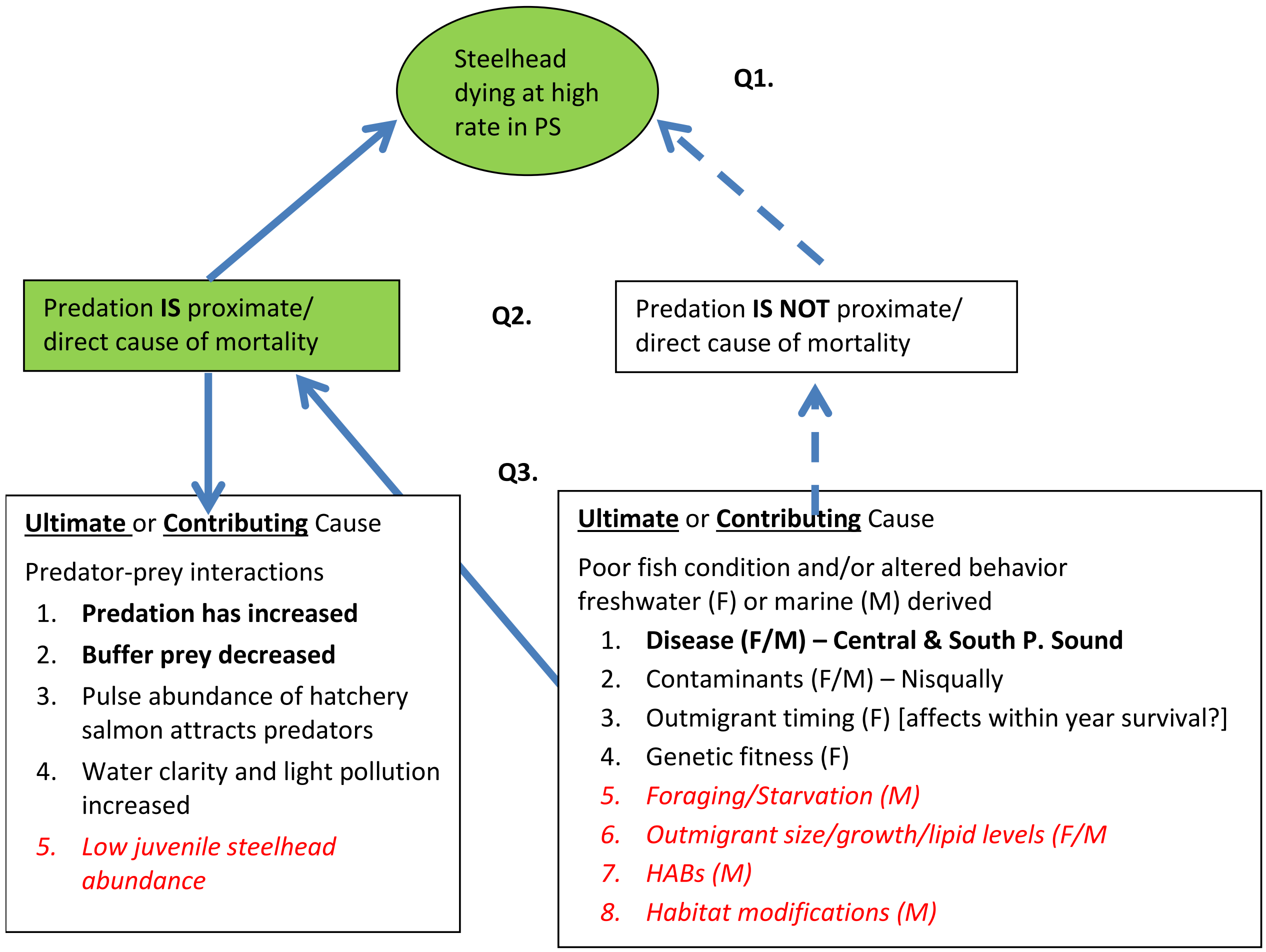


Figure 1. Updated Puget Sound steelhead marine survival logic model. The factors are roughly ranked based upon existing evidence. Those in red have been found to be less likely to contribute to early marine mortality. Q1, 2, and 3 refer back to the three-question framework of the research effort.

Overview of Studies

The following suite of studies is intended to improve our answers to the three questions that constitute the framework of this work plan. These studies were developed by individuals or teams within the Workgroup. See "Appendix A: Study Descriptions" for complete descriptions of each study. See the "Budget and Funding Strategy" section of this report for cost information.

Study 1: Nisqually River PBDE Source Study

A 2014 study on contaminant exposure in outmigrating steelhead trout (*Oncorhynchus mykiss*) from in-river and the estuary habitats of Skagit, Green/Duwamish and Nisqually rivers and their associated nearshore marine habitats documented that polybrominated diphenyl ethers (PBDEs) were highest in the Nisqually River system. Moreover, PBDEs concentrations in steelhead trout were above critical body residues (CBRs) for increased disease susceptibility throughout the Nisqually river system: 33% of fish in-river at the smolt traps, 33% of fish caught in the estuary and 50% of fish in the associated marine basin. Subsequent sampling of steelhead trout at the Nisqually River smolt trap in 2015 also confirmed that approximately one third of the fish had PBDE at levels known to increase disease susceptibility in salmonids. The elevated PBDE levels in steelhead from the Nisqually River was surprising as human development is limited and juvenile Chinook salmon (*Oncorhynchus tshawytscha*) collected from this river were below CBR for increased disease susceptibility. Additionally, because PBDEs were only detected in a portion of the samples, PBDE contaminant exposure is hypothesized to be limited to a subset of the watershed. The purpose of this study is to conduct a source assessment: to identify and prioritize potential sources of PBDE to the Nisqually River so that corrective actions may be implemented. Specific objectives are to: 1) conduct a synoptic survey to assess the spatial distribution of PBDEs in the main stem Nisqually River and its tributaries, and 2) to identify and characterize potential sources of PBDEs to the Nisqually River system, based on the results of the synoptic survey. PBDE concentrations will be measured in water samples (via semi-permeable Membrane SPMDs) and in biofilms (i.e., algae and microbial biomass).

Study 2: Mitigating the Impacts of *Nanophyteus salmincola*

A 2014 study found that prevalence and intensity of *Nanonphyetus salmincola* in steelhead from the Green and Nisqually rivers is high. Prior controlled experiments using steelhead with low-intensity *N. salmincola* infections suggested that the parasite causes deleterious impacts to host survival and swimming performance. These experiments need to be repeated using real-world intensity levels (approximately 10X higher than those used in the aforementioned lab studies). In particular, these controlled experiments will determine lethal parasite loads for steelhead, assess the parasite's impact to swimming performance and determine the parasite's contribution to early marine mortality. Further, in 2016, a qPCR diagnostic tool was developed in order to assess watersheds and hatcheries that are affected by *N. salmincola*. This tool is now being applied to characterize the watersheds and identify *N. salmincola* hotspots so that management options can be considered.

Study 3: Inter-annual and seasonal influences on steelhead smolt survival in Puget Sound

The highest documented steelhead smolt survival through Puget Sound for years 2006-2009 and 2014-2016 occurred in 2016, which also included uncommonly high survival of coho salmon smolts released from Squaxin Island net pens. 2016 was also a year of unusually high anchovy abundance in Puget Sound. Further harbor seal scat sampling in 2016 revealed anchovies contributing to the harbor seal diet during the steelhead and coho smolt outmigrations (Thomas et al. unpublished data). Over the past two decades, the highest anchovy abundance years (Duguid et al. in press) include 2006, 2015 and 2016. These three years coincide with the three highest estimates of early marine survival for steelhead.

A meta-analysis of Puget Sound steelhead telemetry data indicated reduced survival during the first half of May in years of low steelhead early marine survival (Moore et al 2015). This is a period when the Chinook and coho salmon are released into Puget Sound. However, the relationships between steelhead smolt survival rates in Puget Sound and the temporal abundance patterns of hatchery chinook and coho salmon are currently unknown. Hatchery release times do not indicate availability of Chinook and coho salmon to predators in the marine environment because of the added time it takes for these fish to migrate downstream and enter Puget Sound. Therefore, we must capture the time when these Chinook and coho enter the marine waters of Puget Sound. Effects could be positive (prey buffering), negative (predators attracted to areas where steelhead are caused by prey switching), or neutral.

Study 4: Quantifying juvenile salmon and steelhead in harbor seal diet using scat analysis

This study aims to continue to assess Puget Sound harbor seal predation on steelhead and salmon smolts by identifying and quantifying these fish in their diet using hard part and DNA analysis of fecal samples of harbor seals collected during the smolt outmigration window. The percentage of juvenile steelhead and salmon in the harbor seal diet will be estimated based on the co-occurrence of steelhead DNA and juvenile salmonid bones in seal scat samples. These data, combined with quantification of all other prey species, will yield a percentage of seal population global diet comprised of juvenile salmon and steelhead. Those percentages can then be merged with seal bioenergetic data and a population census to estimate the biomass of juvenile salmon and steelhead (or number of individuals) consumed by harbor seals in Puget Sound. Inter-annual changes in the seal global diet will also be assessed as these data may shed light on hypotheses regarding steelhead survival relative to potential prey switching, buffer prey, and pulse abundance of hatchery fish. Scat samples collected in 2017 and 2018 will be processed and analyzed accordingly. Analyses will include the 2016 scat data collected.

Study 5: Relating steelhead characteristics and environmental variables with steelhead smolt survival in Puget Sound and total marine survival trends

Wild and hatchery steelhead individual and population characteristics and environmental data will be analyzed and compared to steelhead smolt-to-adult survival (SAR) trends and survival rates for fish specifically in Puget Sound (derived with acoustic telemetry work) over time. Environmental data will be collected at three spatial scales and include variables such as (but not limited to) those related to river flow, temperature, salinity, turbidity, productivity, upwelling, predators, and buffer prey. The goal of this work is to evaluate the relationship between SAR and Puget Sound survival differences and 1) variation in population life-history diversity and 2) the physical environment of steelhead and conditions therein.

We will examine hypotheses related to spatial variation in mortality, predator-prey interactions, buffer prey, and pulse abundance effects.

TOWARD PUGET SOUND STEELHEAD RECOVERY: CONVERTING THE RESEARCH FINDINGS INTO MANAGEMENT ACTIONS

Through 2018, the Workgroup will continue to work with the Puget Sound Steelhead Recovery Team (Recovery Team) and the Salish Sea Marine Survival Project Coordinating Committee (Coordinating Committee) to incorporate the findings of this research into the Puget Sound Steelhead Recovery Plan. Adaptive management actions and next steps in research, where necessary, will be proposed in the plan based upon the findings. As the results of this research may suggest considerations for marine mammal management[6], hatchery management, disease control, and/or forage fish recovery, we will continue to work with the Recovery Team and Coordinating Committee to include relevant personnel in findings and recovery planning discussions. Finally, results of this study will help inform the forecasting of steelhead population run sizes in the future, improving recovery planning and harvest management.

[6] Recommend including Robert Anderson of NOAA Fisheries and Guy Norman of WDFW for marine mammal management implications.

Project Management, Coordination, Outreach, and Communications

The Workgroup will continue to utilize the project management, coordination, outreach and communications infrastructure of the overarching Salish Sea Marine Survival Project, coordinated by the nonprofit organization Long Live the Kings (LLTK). This will be complemented by WDFW's own outreach and communications capacity.

Project management and coordination

WDFW will lead the implementation of the work plan, and the effort is coordinated by LLTK. As a collaborative effort directly involving NOAA Fisheries, the Nisqually Tribe, US Geological Survey, Seattle City Light, and others, the Workgroup will continue to convene over the course of the study period to plan and implement the research, discuss its outcomes, and determine on what path to continue. Meetings will occur quarterly or more as needed. A project management web site will continue to be used to maintain the research work schedule, communicate regarding activities, and store/manage data.

The Workgroup will coordinate with the Salish Sea Marine Survival Project, Puget Sound Technical Team on overlapping research, research outcomes, and next steps. The Workgroup will also periodically report to the Salish Sea Coordinating Committee on progress and work with the Coordinating Committee and Puget Sound Steelhead Recovery Team on an approach to convert the research results to management actions (see previous section). Under the Salish Sea Marine Survival Project, LLTK will also continue to coordinate this research with the efforts of the Puget Sound Partnership's Puget Sound Ecosystem Monitoring Program. Finally, periodic reports will be provided to the Puget Sound Science Panel who have identified this work as a priority in their Science Plan.

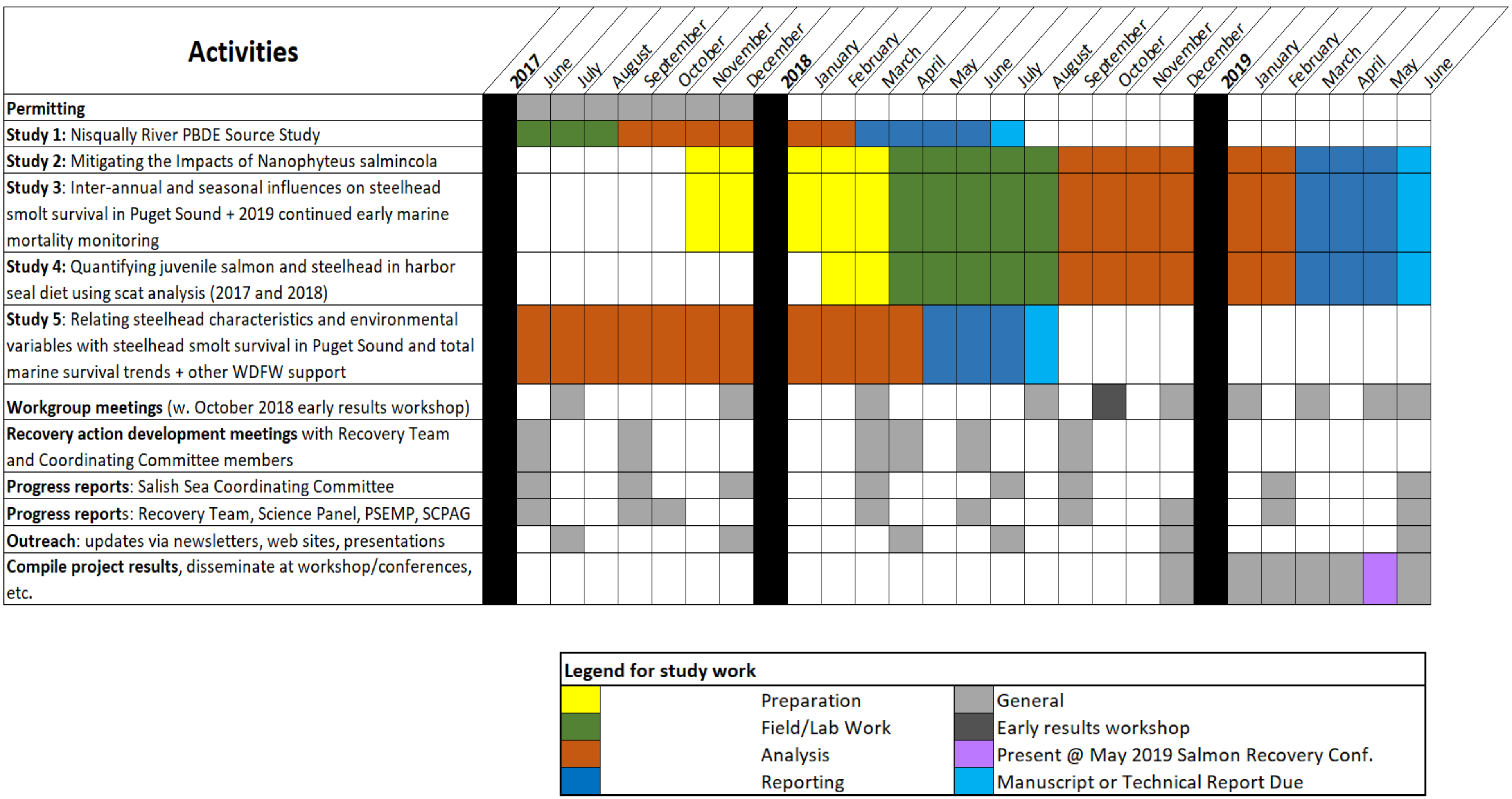
The results of all the studies in the work plan will be comprehensively evaluated by the Workgroup as a whole and will be presented to outside experts in aggregate for review and discussion. This will be led by the WDFW project manager, Neala Kendall, and project coordinator, Michael Schmidt of LLTK. Meetings will be held to disseminate the results and discuss them in aggregate under the umbrella effort, the Salish Sea Marine Project. Also, sessions summarizing the research results will be hosted at conferences or workshops such as the Salmon Recovery Conference, the Salmon Ocean Ecology Workshop, the Salish Sea Ecosystem Conference, and the biennial Pacific steelhead management meeting hosted by the Pacific States Marine Fisheries Commission. See the Work Schedule section, below, for more information.

Outreach and communications

The outreach and communications effort will includes updates on the Salish Sea Marine Survival Project's public web site, WDFW weekender reports, LLTK newsletters, presentations to the Project Coordinating Committee, and periodic presentations to the local sport fishing groups including WDFW's Steelhead and Cutthroat Policy Advisory Group (SCPAG), WDFW's Puget Sound Recreational Fisheries Enhancement Oversight Committee, and Puget Sound Anglers. As we have in the past, over the long-term LLTK will also work with local news groups to report on study findings and the results of certain management actions.

WORK SCHEDULE

The following diagram describes the workflow. Coordination and outreach activities are included to describe how progress and results will be communicated. All work is completed by June 30th, 2019.



Budget and Funding Strategy

The following is a general budget for the 2017-2019 research phase. The total cost of the effort is ~$900,000. Current revenue is being provided via a Washington State Appropriation, the Nisqually Indian Tribe, US Geological Service and the SeaDoc Society. In-kind match in staff time is not fully accounted for in this budget. Funding decisions were made based upon research priorities.

2017-2019 Puget Sound Steelhead Marine Survival Budget	
Expenses	**Amount**
Project management and coordination	$70,000
Study 1: Nisqually River PBDE Source Study	$41,251
Study 2: Mitigating the Impacts of Nanophyteus salmincola	$195,183
Study 3: Inter-annual and seasonal influences on steelhead smolt survival in Puget Sound + 2019 continued early marine mortality monitoring	$241,885
Study 4: Quantifying juvenile salmon and steelhead in harbor seal diet using scat analysis (2017 and 2018)	$221,022
Study 5: Relating steelhead characteristics and environmental variables with steelhead smolt survival in Puget Sound and total marine survival trends + other WDFW support	$115,483
Total	*$884,824*
Revenue	**Amount**
Washington State Appropriation	$748,390
Nisqually Indian Tribe	$51,251
USGS	$40,000
SeaDoc Society	$45,183
Total	*$884,824*

APPENDIX A: STUDY DESCRIPTIONS

Study 1: Nisqually River PBDE Source Study

Investigators: Sandra O'Neill[1], William Hobbs[2], Laurie Niewolny[1], Andrea Carey[1] and Sayre Hodgson[3]

[1]*Washington Department of Fish and Wildlife*

[2]*Department of Ecology*

[3]*Nisqually Indian Tribe*

Summary

A 2014 study on contaminant exposure in outmigrating steelhead trout (*Oncorhynchus mykiss*) from in-river and the estuary habitats of Skagit, Green/Duwamish and Nisqually rivers and their associated nearshore marine habitats documented that polybrominated diphenyl ethers (PBDEs) were highest in the Nisqually River system. Moreover, PBDEs concentrations in steelhead trout were above critical body resides (CBRs) for increased disease susceptibility throughout the Nisqually river system: 33% of fish in-river at the smolt traps, 33% of fish caught in the estuary and 50% of fish in the associated marine basin. Subsequent sampling of steelhead trout at the Nisqually River smolt trap in 2015 also confirmed that approximately one third of the fish had PBDE at levels known to increase disease susceptibility in salmonids. The elevated PBDE levels in steelhead from the Nisqually River was surprising as development is limited and juvenile Chinook salmon (*Oncorhynchus tshawytscha*) collected from this river were below CBR for increased disease susceptibility. Additionally, because PBDEs were only detected in a portion of the samples, PBDE contaminant exposure is hypothesized to be limited to a subset of the watershed. **The purpose of this study is to conduct a source assessment study to identify and prioritize potential sources of PBDE to the Nisqually River so that corrective actions may be implemented.** Specific objectives are to 1) conduct a synoptic survey to assess the spatial distribution of PBDEs in the main stem Nisqually River and its tributaries, and 2) to identify and characterize potential sources of PBDEs to the Nisqually River system, based on the results of the synoptic survey. PBDE concentrations will be measured in water samples (via semi-permeable Membrane SPMDs) and in biofilms (i.e., algae and microbial biomass).

Objectives

Based on the results of the 2014 and 2015 studies, we hypothesized that PBDE contaminant exposure is limited to a subset of the in-river habitat, because only a portion of the steelhead samples at smolt trap in both years had PBDE concentrations above CBR for increased disease susceptibility. Uniform contaminant exposure among individuals steelhead collected at the trap would be expected if the PBDE contaminant sources were wide-spread throughout the in-river habitat.

Understanding the sources of PBDE exposure for steelhead trout originating from the Nisqually River is necessary to identify and prioritize corrective management actions that may increase their survival. <u>The purpose of this study is to conduct a source assessment to identify and prioritize sources of PBDE to the Nisqually River.</u> WDFW's T-BiOS team, in collaboration with the Department of Ecology (Ecology), will collect co-located water and biofilm samples to identify PBDEs present in the water across diverse ecological sites throughout the Nisqually River watershed. Specific objectives are to 1) conduct a synoptic survey to assess the spatial distribution of PBDEs in the main stem Nisqually River and its tributaries, and 2) to identify and characterize sources of PBDEs to the Nisqually River system, based on the results of the synoptic survey.

Study Design

Sampling Design

Water and biofilm samples will be collected at strategic locations throughout the Nisqually River and at select tributaries to identify sources of PBDEs to the system, based on potential PBDE inputs. Water samples will be collected with semipermeable membrane devices (SPMDs), a type of passive sampler. Biofilm samples, an aggregation of periphyton, microbes, and fine sediment scraped off rocks, will be collected at sites in the immediate vicinity of passive water sampling sites, to the extent possible. Additionally, water grab samples will be collected and analyzed for ancillary water quality parameters (i.e., dissolved and total organic carbon and suspended solids) to help understand relationships between suspended matter and the PBDE contaminants.

The sampling locations include two sites along the mainstem Nisqually River, one site and confluence of Centrailia Canal and the Nisqually River and three major tributaries, Muck Creek, Ohop Valley Creek, and Mashel River (Figure 1), which collectively encompass know potential inputs of PBDEs to the system.

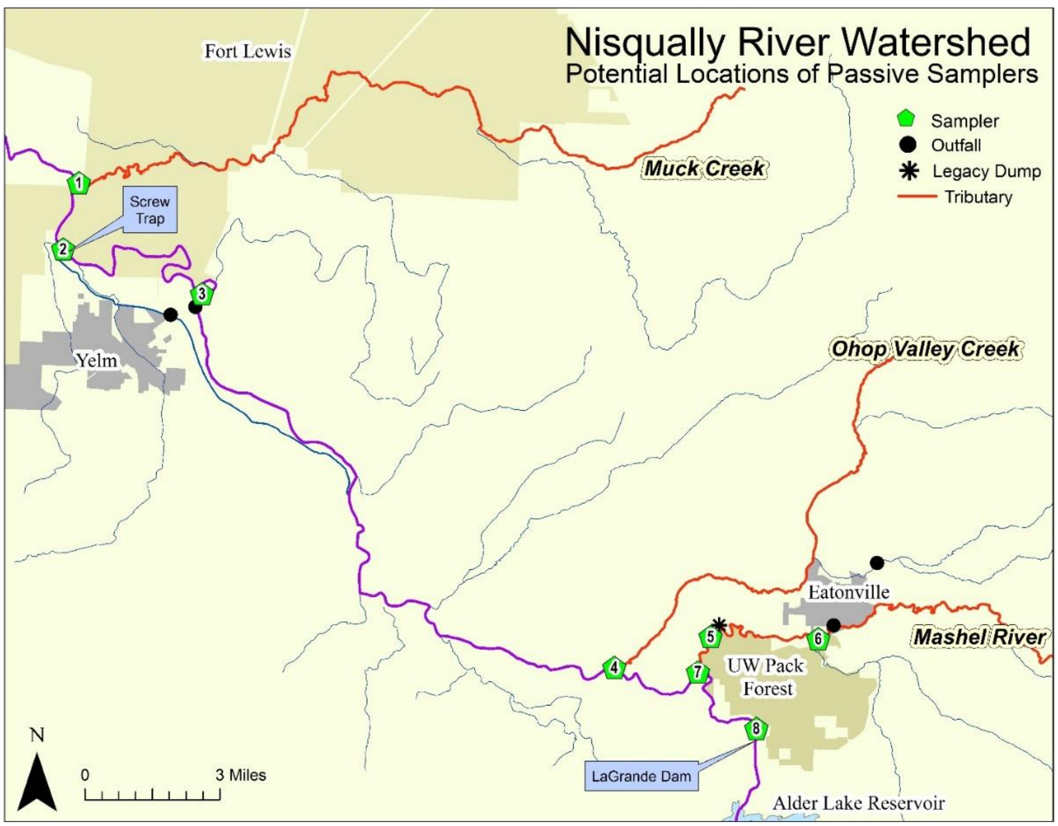


Figure 2 Map of the Nisqually River watershed noting the mainstem (colored purple,) major tributaries (colored red), major WWTP and stormwater outfalls, a legacy dumpsite, the major land use facilities/land (i.e., Fort Lewis and UW Pack Forest) and the proposed locations of passive water samplers to measure PBDEs in water.

WDFW and Nisqually Tribal staff identified potential sources of PBDEs to the Nisqually River watershed in the planning phase for this project, based on historical and current land uses (Table 1). Specific potential sources include three wastewater treatment plant (WWTP) outfalls, a major stormwater outfall, and surface stormwater runoff from a former (i.e. legacy) dump used by Weyerhauser, the Fort

Lewis facility/lands, and the University of Washington's (UW) Charles L. Pack Experimental Forest research facility/lands (UW Pack Forest). Additionally, diffuse runoff from agricultural, residential and forested lands within the Ohop Valley Creek and Mashel River watersheds could potentially input PBDE to the Nisqually river-system, although no explicit sources have been identified.

Table 1. Specific, potential sources of PBDEs to the Nisqually River watershed.

Potential PBDE source	Location of Potential Inputs
Fort Lewis facilities/lands	associated watershed flows to Muck Creek
City of Yelm-Centralia Canal WWTP outfall	discharges to Centralia Canal
City of Yelm WWTP outfall	discharges to mainstem Nisqually River
Eatonville stormwater outfall	discharges to a tributary of Ohop Valley Creek
City of Eatonville WWTP outfall	discharges to Mashel River, just below Eatonville
Legacy dump (used by Weyerhauser)	associated watershed flows to Mashel River
UW Pack Forest - occasional application of sewage sludge as an experimental fertilizer	associated watershed flows to Mashel River
Atmospheric deposition to upper watershed	flows to Alder Lake, above LaGrande Dam

Our sampling design will investigate whether the source of PBDEs to the Nisqually river-system is from inputs to one of the three main tributaries, the Centralia Canal, the mainstem, or a combination of these inputs. Table 2 lists a description of the sampling sites and the potential PBDEs sources to each site.

A SPMD will be placed in Muck Creek, just above the confluence with the mainstem Nisqually River, to assess potential PBDE inputs from surface stormwater runoff from the Fort Lewis facility/lands, although we are not aware of any known PBDE sources. Muck Creek flows through the southeastern section of the 87,000-acre Fort Lewis facility/lands, a military complex consisting of Joint Base Lewis-McChord, a training and mobilization center for all the armed services.

Another SPMD will be placed in the Ohop Valley Creek just above the confluence of the mainstem Nisqually River to assess potential PBDE inputs to the mainstem Nisqually River from the Ohop Valley Creek. The Ohop Valley Creek receives stormwater from a City of Eatonville via an outfall that is located on a tributary of Ohop Valley Creek. Additionally, the Ohop Valley Creek watershed consists of mixed uses (agriculture, residential properties, and forests) that may contribute inputs of diffuse stormwater surface runoff of PBDEs.

Table 2

Sample Location Number in Figure 1	Location Description	Nisqually River Mile	Tributary Mile	Potential PBDE Inputs Sampled
1	Muck Creek, just before the confluence with the Nisqually River	10	0.1	Fort Lewis facility/lands

2	Centralia Canal, immediately upstream of where it the flows into Nisqually River	11.7	0.01	City of Yelm-Centralia Canal WWTP outfall
3	Nisqually River mainstem, adjacent to Yelm	18.7	NA	City of Yelm WWTP outfall and other upstream inputs
4	Ohop Valley Creek, just before the confluence with the Nisqually River	34.1	0.1	Eatonville stormwater outfall and other unknown inputs on Ohop Valley Creek.
6	Mashel River, just below Eatonville	37.1	4.9	City of Eatonville WWTP outfall & other unknown inputs upstream
5	Mashel River, downstream of Eatonville and legacy dump and midway of UW Pack Forest	37.1	1.3	runnoff from legacy dump (Weyerhauser/City of Eatonville), and portion of UW Pack Forest , Eatonville WWTP outfall, and unknown inputs on the Mashel River
7	Mashel River, just before the confluence with the Nisqually River	37.1	0.1	runnoff from legacy dump (Weyerhauser/City of Eatonville), and portion of UW Pack Forest , Eatonville WWTP outfall, and unknown input on the Mashel River
8	Nisqually River mainstaim, just downstream of LaGrande dam	40.1	NA	atmospherically deposited PBDEs to upper Nisqually River watershed

Three SPMDs units will be placed in the Mashel River, which supports 50% of the steelhead production in the Nisqually River system. The furthest upstream SPMD will assess inputs from the City of Eatonville's WWTP, and will be placed just downstream of the outfall. The second SPMD will be placed just downstream of a legacy dump used by Weyhauser, and alongside UW's PAC Forest) to assess potential inputs in surface stormwater runoff from these sites. Surface water runoff from the legacy dump could contain PBDE leaching from previously dumped furniture and electronics. PAC Forest encompasses 4,300 acres of working forestland and has occasionally used sewage sludge as an experimental fertilizer, a possible source of PBDEs. The third SPMD will be placed just upstream of the confluence of the Mashel River and the confluence of the mainstem Nisqually River and would assess multiple inputs from the Mashel River (i.e., Eatonville WWTP outfall, stormwater runnoff from legacy dump (Weyerhauser/City of Eatonville), and portion of UW Pack Forest , and other unknown input on the Mashel River).

In addition to sampling the waters of the three major tributaries, waters of the Centralia Canal will be sampled. An SPMD will be placed immediately upstream of the confluence of the Central Canal and the mainstem Nisqually River to assess inputs from the Yelm-Centralia Canal WWTP outfall that discharges at this site.

Two SPMDs will be placed along the mainstem Nisqually River, one sited just below the La Grande Dam on the upper most reach of the Nisqually River (river mile 40.1) and the other adjacent to city of Yelm (river mile 18.7). The SPMD below the La Grande Dam will assess if the upper mainstem is a PBDE source, primarily through atmospheric deposition of PBDEs in snowmelt and precipitation from Mt. Rainier and the surrounding area. The SPMD adjacent to the City of Yelm will assess inputs from Yelm's WWTP outfall and other cumulative upstream inputs.

Based on Puget Sound loading studies (Ecology and King County,2011) inputs from WWTPs are a more likely source sources of PBDEs than input from stormwater outfalls or surface stormwater runoff. Accordingly, sampling for this project will take place during the low flow period at the end of the summer (i.e. September) when inputs from WWTPs are concentrated in the river water, improving our chances of detection of PBDEs.

Preparation and Deployment of semi-permeable membrane devices (SPMDs)

SPMDs are composed of a thin-walled, layflat polyethylene tube (91.4 cm x 2.5 cm x 70-95 um thickness) filled with 1 ml of triolein, a neutral lipid compound (Figure 2). The goal of any passive sampling device is to emulate natural biological uptake by allowing chemicals to diffuse through the membrane and concentrate over time (typically a 28-day deployment). After deployment, the membranes are removed, extracted, and analyzed for the contaminant of interest.

Figure 2: An SPMD canister showing the upper membrane. Some biofouling on the membrane is evident.

In this study, SPMDs will be deployed in secure areas (i.e., to minimize vandalism and avoid strong currents), using stainless steel canisters and spindle devices provided by Environmental Sampling

Technologies (EST). To guard against possible loss of canisters/SPMDs, two canisters/SPMDs will be placed at each site however, only one will be analyzed for the presence of PBDEs. The second canister/SPMDs are backups that would only be analyzed if the other canister/SPMD at the site is lost. Each site canister/SPMD will contain five membranes preloaded onto spindles by EST, and shipped in solvent-rinsed metal cans under argon gas. Prior to deployment, performance reference compounds (PRCs) will be spiked into the membranes in order to assess biofouling and the non-equilibrium uptake of the compounds of interest (Huckins et al., 2006). The use of PRCs is essentially an *in situ,* site-specific calibration technique based on the observation that the rate of residue loss is proportional to the rate of residue uptake. The labeled congener (PBDE-138L will be used as PRCs, where “L” denotes a 13C labeled compound. PRCs will be added at a concentration of 2.5 ng per SPMD.

A StowAway® TidbiT™ temperature logger will be attached to each canister to continuously monitor the water temperature during deployment. A second data logger will be attached nearby to monitor air temperature. The data collected from the temperature loggers will be used to confirm that the SPMD remained submerged during the sampling period.

SPMDs will be exposed for no more than 45 seconds at each site during deployment and retrieval. Nitrile gloves will used at all times. SPMDs will be deployed for approximately 28 days in the late summer (i.e. September), when water flows are low. The same cans will be used during retrieval. They will be properly sealed, cooled, and kept near freezing until arrival at AXYS Analytical for the extraction of the membranes (dialysis). PBDE analysis will be performed by AXYS via EPA Method 1614, AXYS method MLA-033.

Collection and Analyses of Biofilm

Biofilm refers to the mixture of periphyton, microbial biomass, and fine sediments. Periphyton is algae attached to the river bottom, rocks, or debris in the river. Standard protocols will be followed for sampling attached algae (Stevenson and Bahls, 1999; Mathieu et al., 2013). Biofilm will be scraped from rocks and collected in a stainless bowl for weighing in the field to confirm that sufficient biomass is retrieved. Samples will be transferred from the bowl to a cleaned glass jar. A sample to assess areal biomass (g dry weight / cm^2) will be collected separately; each rock scraped for biofilm will be measured by cutting a piece of aluminum foil tracing the sample area. The aluminum foil is then measured at Ecology using the Image J software.

Figure 3: Example of a biofilm being scraped from a rock.

Biofilms will be analyzed for PBDEs, ash-free dry weight (areal biomass), and carbon (C) and nitrogen (N) abundance and stable isotope ratios. Stable isotopes of the biofilms will assist in detecting changes in nutrient and wastewater inputs over the study area.

Surface water grab samples

Water grab samples will be taken to measure the total and dissolved organic carbon (TOC/DOC) and suspended solids (SS) each site during the time the SPMDs are exposed. These parameters will be used as ancillary data to help understand relationships between suspended matter and the PBDE contaminants. Water grab samples will be sampled three times over the duration of the SPMD exposure to get an integrated measure of the conditions. Grab samples will be collected using Ecology standard operating procedures (Joy, 2006).

Additional field parameters will be measured *in situ* at the time of water sampling using a Hydrolab DataSonde (Swanson, 2007). Parameters include: temperature, pH, dissolved oxygen, and conductivity.

Tasks by organization

WDFW will lead scoping and planning efforts and securing analytical services for PBDEs. Ecology will provide assistance and logistical support specifically, but not exclusively, securing SPMDs and the sampling equipment, assisting with deployment and retrieval, data analysis, and reporting. Both organizations will continue to communicate to identify critical tasks for deployment to occur in the month of September. Most important is the ordering of SPMDs and standards to take place in

July and August. Deployment and retrieval activities will be staffed cooperatively dependent on other field activities. Data analysis and reporting of results will be a mutual activity depending on the expertise needed.

Outcomes

The results of this work will be a technical report that identifies and characterize sources of PBDEs to the Nisqually River system most likely affecting the wild steelhead there.

Timeline

Table 3. Tentative Timeline

Month	Action
July 2017	Planning-finalize work plan
August 2017	Secure equipment and services/ prepare samples
September 2017	Field sampling (deploy)
October 2017	Field sampling (retrieve)
February 2018	Chemical data receipt
March to May 2018	Data analysis and reporting

Deliverables

WDFW and DOE will provide a technical report describing the results of the work.

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Study 2: Mitigating the Impacts of *Nanophyetus salmincola*

Investigators: Paul Hershberger[1]

[1]*US Geological Survey*

Summary

Nanophyetus salmincola is a digenean trematode that infects wild and propagated salmonids, including federally protected steelhead. The direct and indirect impacts of *N. salmincola* in the Puget Sound region are increasingly observable. For example, recent surveys documented 100% infection prevalence in outmigrating wild steelhead from the Nisqually River, with mean parasite loads occurring well-above lethal intensities. Early marine survival and smolt-to-adult return rates of steelhead are lower in *N. salmincola* -positive watersheds from southern Puget Sound than in *N. salmincola* -free watersheds from northern Puget Sound and the northern Washington coast. In Washington State, some salmon enhancement facilities (e.g. McAllister Creek Hatchery and Johns Creek Hatchery) have been decommissioned as a result of unmanageable mortality from *N. salmincola* . Other enhancement facilities (e.g. Soos Creek Hatchery) are responding to the disease emergence using unsustainable management strategies, including the implementation of sacrificial fish embryos as *N. salmincola* biofilters.

N. salmincola has a complex life cycle that involves a freshwater snail (*Juga* spp.) as the first intermediate host, freshwater and anadromous fishes as the second intermediate host, and birds / terrestrial mammals as the definitive host. For this reason, the parasite's freshwater range from northern California through central Washington is determined primarily by the distribution of the snail intermediate host.

Recognizing the importance of the waterborne cercaria stage that is released from the snail and becomes infectious to fishes, a quantitative polymerase chain reaction (qPCR) assay was recently developed; the assay is capable of detecting a single *N. salmincola* cercaria from a water sample. This novel tool was recently applied to document the seasonality cercaria shedding and deduce the timing of fish exposures to the parasite within an affected watershed.

Additional research on this parasite is required to develop management strategies capable of mitigating the impacts of the resulting disease to wild and propagated salmonids.

Objectives and Brief Overview of Study Concepts

The objectives of proposed research during 2018-2019 include:

<u>Determine peak periods of cercaria shedding throughout the day</u>. Prior research documented the annual seasonality of cercaria shedding within an affected watershed; however, follow-up work needs to be performed using the newly-developed qPCR to determine whether exposure periods peak during certain periods throughout the day.

<u>Determine where *N. salmincola* hot spots exist within an affected watershed</u>. Using the newly-developed qPCR, follow-up work needs to be performed to identify the *N. salmincola* hot spots within a positive watershed. The Nisqually River will be characterized. Documentation of the existence of these

sites could offer future mitigation strategies for wild and propagated fishes. Further, the habitat, including riparian forest cover, will be characterized in hotspots vs not as it may provide support for specific mitigation actions (Hypothesis = deciduous cover provides more spring/fall seasonal light, promoting nano blooms, and leaf material as food promotes snail growth).

Determine effects of *N. salmincola* infections to steelhead health and survival. Prior controlled experiments using steelhead with low-intensity infections suggested that the parasite causes deleterious impacts to host survival and swimming performance. These experiments need to be repeated using real-world intensity levels (approximately 10X higher than those used in the aforementioned lab studies). In particular, these controlled experiments will determine lethal parasite loads for steelhead, document the progression of the invading cercaria through the fish tissues, and assess the effects of infections on host swimming performance.

Determine the effects of *N. salmincola* on the early marine survival of steelhead smolts. A prior experiment, using hydroacoustic tags in infected and uninfected steelhead, indicated that low-intensity *N. salmincola* infections may negatively influence early marine survival. This experiment needs to be repeated using real-world parasite loads (approximately 10X higher than those used in the aforementioned field study). Briefly, two groups of steelhead (infected and uninfected) will be created in the laboratory; both groups will receive hydroacoustic tag insertions and will then be released into Puget Sound. Passage of these fish through receiver arrays located in Puget Sound will be used to deduce the relative survival between the two groups.

Timeline

Field sampling will occur in spring 2018. Analysis will follow, through December 2018. Reporting will occur between January and June 2018.

Deliverables

At least one manuscript and one technical report will be developed describing the findings of this effort. The manuscript will describe the direct and indirect effects of *N. salmincola* on steelhead smolt survival and the technical report will describe the hotspots for *N. salmincola* in the Nisqually River.

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Study 3: Inter-annual and seasonal influences on steelhead smolt survival in Puget Sound

Investigators: Barry Berejikian[1], Steve Jeffries[2], Megan Moore[1], Jed Moore[3], Matt Klungle[2], Steve Rubin[4], Phillip Dionne[2]

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Summary

The highest documented steelhead smolt survival through Puget Sound for years 2006-2009 and 2014-2016 occurred in 2016, which also included uncommonly high survival of coho salmon smolts released from Squaxin Island net pens. 2016 was also a year of unusually high anchovy abundance that was associated with documented major foraging events involving sea lions, harbor seals, and sea birds and. High anchovy abundance may have sufficiently occupied potentially significant predators of steelhead during their spring migration. Harbor seal scat sampling in 2016 revealed a significant contribution of anchovies to the harbor seal diet during the steelhead and coho smolt outmigrations (Thomas et al. unpublished data). Evidence of high anchovy abundance comes from several different sources, which individually do not provide high resolution on inter-annual variation in anchovy biomass, but taken together, provide a relative ‘high vs low’ signal for anchovies in the Salish Sea (Duguid et al in press). Over the past two decades, the highest anchovy abundance years include 2006, 2015 and 2016. These three years coincide with the three highest estimates of early marine survival for steelhead.

A meta-analysis of Puget Sound steelhead telemetry data indicated reduced survival during the first half of May (Moore et al 2015), which is when the majority of Chinook and coho salmon are released into Puget Sound. For example, approximately 20 milliion hatchery Chinook salmon are released into South/Central Puget Sound each spring mostly initiated during the peak of the wild steelhead smolt migration. However, the relationships between steelhead smolt survival rates in Puget Sound and the temporal abundance patterns of hatchery chinook and coho salmon are currently unknown. Effects could be positive (prey buffering), negative (caused by prey switching) or neutral. Furthermore, release times do not indicate availability of Chinook salmon to predators in Puget Sound. Release dates differ among hatcheries and travel times from release locations to river mouths will likely differ by migration distance and local river conditions (flow and temperature).

Thus, there are two non-mutually exclusive hypotheses related to predator-prey interactions: 1) the anomalous anchovy pulse in 2016 caused a significant overall increase in the survival of steelhead and coho salmon smolt relative to the other 6 years in the telemetry dataset; 2) pulses of hatchery salmon affect steelhead survival within a migration season on temporal scales of one to several weeks. The approach is to build on existing datasets to further inform these hypotheses, since neither can be tested in a controlled experimental fashion.

Objectives and Brief Overview of Study Concepts

Task 1: Conduct an acoustic tagging and monitoring program for steelhead to estimate survival rates through Puget Sound and in relation to above factors (2018 -2019).

From late April through early June (7 weeks total), wild steelhead smolts (n = 210 in 2018; n = 100 in 2019) would be captured at the Nisqually River screw trap, implanted with acoustic transmitters and released. Hydrophones would be placed in the same areas as in previous investigations in 2014 and 2016 (Nisqually estuary, pilings, at harbor seal haulouts, and the NAR, CPS, ADM, and JDF arrays). Mobile tracking would occur over the same transects as in previous years and would occur in late June/early July (see Table 1 for summary of past and planned hydrophone deployments).

Steelhead smolts will be captured at the WDFW rotary screw trap location in the Nisqually River. Captured smolts will be held for one day before being anesthetized, weighed (Moore et al. 2015), measured (fork length) and implanted with a Vemco V7 2L acoustic transmitter (Vemco, Nova Scotia, Canada). Surgical implantation procedures are described in (Moore and Berejikian 2017). A total of 210 smolts will be tagged over a seven-week period beginning in late April. Tags will emit an acoustic ping (136 db) every 30 – 90 s on a random delay cycle. All smolts will be held for approximately 24 hours before being transported and released at river kilometer 19.

Six stationary Vemco VR2W receivers will be deployed in the Nisqually estuary. Tags will also be detected at four additional Vemco VR3 receiver arrays: 20 km north of the Nisqually River (near the Tacoma Narrows; 8), 20 km north of the Green River (in Central Puget Sound 19 VR3 receivers), in Admiralty Inlet (13 VR3 receivers), and at the western end of the Strait of Juan de Fuca (30 VR3 receivers; maintained by the Ocean Tracking Network)

Haulouts in south, central and north Puget Sound will be monitored with fixed Vemco VR2W receivers. Multiple receivers will be anchored near each haulout capture location to detect the movements of tagged smolts near the haulout areas. These receivers will provide i) known locations of tags, ii) indications of whether the behavior of tags is different near haulout areas than at other monitored sites (e.g., Estuary, Narrows, CPS, Admiralty arrays), iii) indications of tags that may have been consumed and carried by harbor seals. Differences in the behavior of tags at haulout sites compared to non-haulout locations (fixed receiver arrays) will allow inferences regarding the proportion of tags at haulouts that are still in live, migrating steelhead and those that have been consumed and are being carried by a predator.

After the smolt outmigration period (mid-June), a boat-mounted Vemco VR-100 mobile receiver will be be deployed throughout Puget Sound to identify the locations of stationary tags at predetermined locations that replicates effort from past years. This will provide a more extensive spatial description of the fate of steelhead tags in areas not monitored by the fixed arrays or frequented by the seal-mounted VMTs.

Task 2: Estimate marine entry timing of Chinook and coho salmon from South and Central Puget Sound Hatcheries (2018).

The proposal here is to sample specific areas in South and Central Puget Sound to quantify peak marine entry timing of released hatchery Chinook and coho salmon in nearshore habitats adjacent to 1) the Nisqually estuary, 2) Commencement Bay/Puyallup River Estuary, 3) Elliott Bay/Duwamish River Estuary, and 4) Shilshole Bay/Lake Washington and Lake Union outlet. Other hatcheries in South and Central Puget Sound also release large numbers of hatchery Chinook and coho salmon (e.g., Deschutes, Minter Creek, and Grovers Creek), but these are essentially tidewater release sites and marine entry timing can be more closely approximated by release time.

The Nisqually Tribe and US Geological Survey, Western Fisheries Research Center have collaborated on studies using lampara nets to capture hatchery Chinook and coho salmon in the Puget Sound nearshore environment near the Nisqually Reach, and have been successful at quantifying the temporal and spatial distribution of released hatchery-reared salmon (Hodgson et al. 2016). For example, the 'nearshore-cormorant' sampling locations yielded the highest catch rates near the Nisqually estuary and sampling should focus on this area. Specific sampling areas near the other three river mouths will need to be identified.

For this effort, sampling using lampara nets would occur from mid-April through mid-June. Samplers will conduct a full day of sampling (20 sites and one set per site) in each of the four locations each week. Samplers will aim for Tuesday-Wednesday sampling each week, with Monday as a planning day and Thursday/Friday as a back-up for weather/gear/personnel issues. Planning documents have been created to identify for each agency, the number of boats and staff available for each day from April 16 through June 15. Each sampling effort will involve the use of two boats and a minimum of two crew members per boat. Three crew members per boat where capacity exists would be preferable and should be a goal during planning/scheduling efforts, especially where there are expectations that additional information or sampling is requested by potential collaborators. See separate netting and fish sampling protocols document for capture/handling procedures. One set at each of 20 sites per area will be conducted. Sites will be prioritized, and highest-priority sites will be sampled first. Catch per set averaged over pre-determined transects within a sampling location will be used to profile the onset and peak abundance of both natural and hatchery origin smolts entering Puget Sound.

Task 3: Conduct scat sampling and diet analysis to estimate harbor seal diet fractions over time (2018).

See Study 4, below.

Table 1. Temporal depiction of tagging, salmon collections, hydroacoustic surveys and scat collections in South and Central Puget Sound

Activity	April 1-15	April 16-30	May 1-15	May 16-31	June 1-15	June 16-30
Steelhead tagging		x	x	x	x	
Lampara net		x	x	x	x	
Scat collections	x	x	x	x	x	x

Table 2. Past and proposed hydrophone deployments, scat collections, and smolt tagging.

Acoustic Telemetry Hydrophone deployments						Sampling	
Year	VR2 Nisqually Estuary	VR3 Arrays	VR 100 Mobile tracking	Seal mounted VMT	Nearshore VR2	Scat collections	Smolts tagged
2014	6	NAR, CPS, ADM	No	Yes	SS		143
2015	6	NAR, CPS, ADM	Yes	No			100
2016	6	NAR, CPS, ADM	Yes	Yes	SS to ADM	Yes	200
2017	6	NAR, CPS, ADM	Yes	No		Yes	100
2018	7	NAR, CPS, ADM	Yes	No	SS to ADM	Yes	210
2019	7	NAR, CPS, ADM	Yes	No		None planned	100

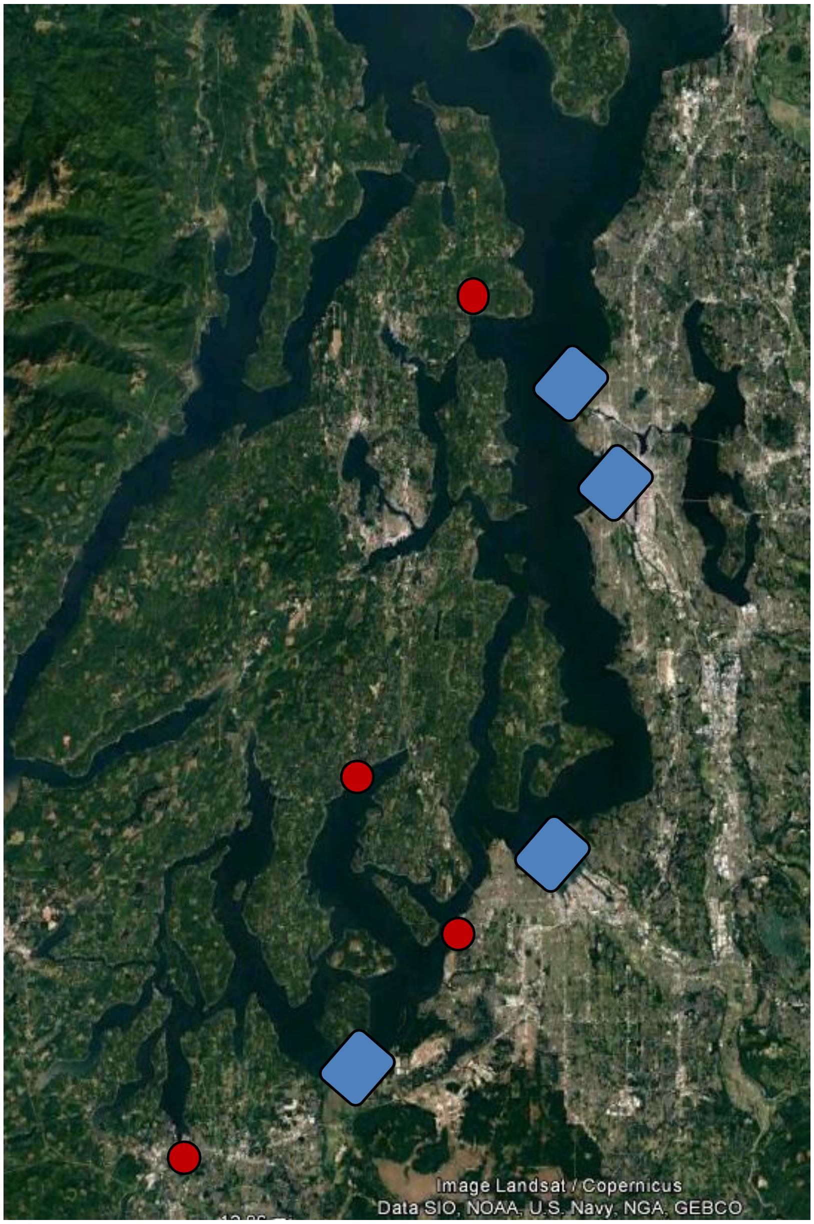

Figure 1. Proposed general locations (blue rectangles) for lampara netting to determine marine entry timing for Chinook and coho salmon. Red dots represent near-tidewater hatcheries where marine entry timing will be estimated from release date information.

Timeline

Field sampling will occur in spring 2018. Analysis will follow, through December 2018. Reporting will occur between January and June 2019.

Deliverables

At least one manuscript will be developed describing the findings of this effort related to alternative prey (buffer or swamping) and prey switching hypotheses.

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Study 4: Quantifying juvenile salmon and steelhead in harbor seal diet using scat analysis

Investigators: Steve Jeffries[1], Monique Lance[1], Ken Warheit[1], Austen Thomas[2], Jed Moore[3], Bill Walker[4]

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Summary

This study aims to continue to assess harbor seal predation on smolts by identifying and quantifying juvenile steelhead and salmon in their diet using hard part and DNA analysis of fecal samples of Puget Sound harbor seals collected during the outmigration window. The percentage of juvenile steelhead and salmon in the harbor seal diet will be estimated based on the co-occurrence of steelhead DNA and juvenile salmonid bones in seal scat samples. These data, combined with quantification of all other prey species, will yield a percentage of seal population global diet comprised of juvenile salmonids and steelhead.

Those percentages can then be merged with seal bioenergetic data and a population census to estimate the biomass of juvenile salmon and steelhead (or number of individuals) consumed by harbor seals in South Puget Sound. Inter-annual changes in the seal global diet will also be assessed as these data may shed light on hypotheses regarding steelhead survival relative to potential prey switching, buffer prey, and pulse abundance of hatchery fish. Scat samples collected in 2017 and 2018 will be processed and analyzed accordingly. Analyses will include the 2016 scat data collected. If agreed to by the collaborators, the seal diet data from this study will be published along with seal diet data collected from the Strait of Georgia since 2011 in an open data access journal.

Objectives

Objective 1 – Obtain direct evidence of harbor seal steelhead predation in Puget Sound using scatological analysis of harbor seal fecal samples.

Objective 2 – Quantify the percentage of harbor seal population diet comprised of salmonids including steelhead in Puget Sound, producing data useful for estimating the numbers of juvenile and adult salmon and steelhead consumed by seals.

Objective 3 – Compare scat-based estimates of harbor seal predation from scats with earlier diet studies to detect and evaluate anchovy/forage fish/hatchery smolts relationships.

Study Design

Scat sampling

Harbor seal haulout sites in southern Puget Sound will sampled for the purposes of scat collection and estimating prey consumption.

Scats will be collected every other week between late-March and mid-June, for a total of 6 collections, targeting low-tide temporal windows when appreciable numbers of scats can be acquired. We will strive to collect 70-90 harbor seal scat samples from seal haulout sites in southern Puget Sound during each biweekly collection trips. This sample size is a rule of thumb determined from a statistical power analysis for seal and sea lion diet studies (Trites& Joy 2005).

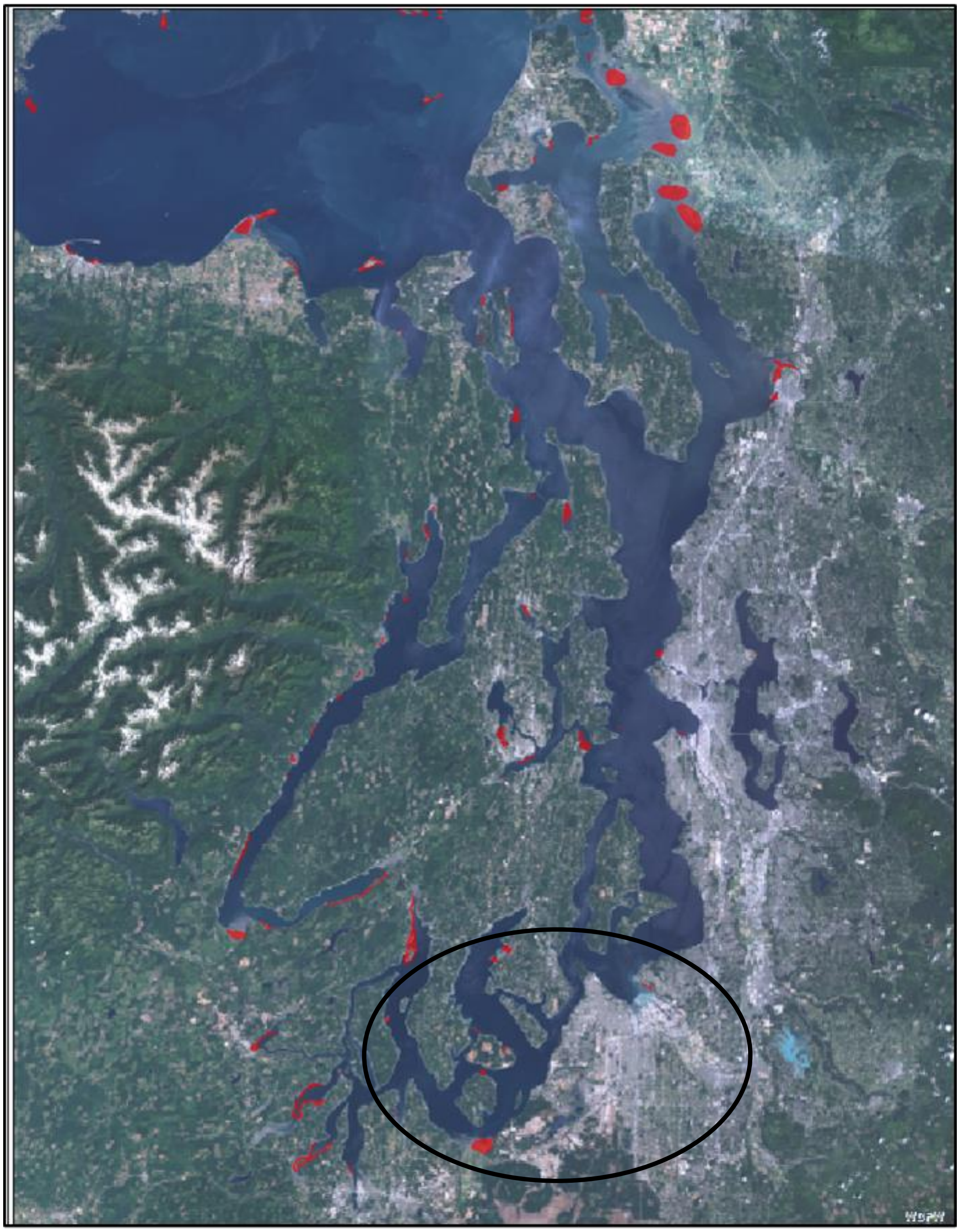

Figure 1. Study area depicting seal haulout areas where scat samples can be collected.

At the haulout sites, each individual scat sample will be collected using a disposable wooden tongue depressor and placed in a gallon ziplock type plastic bag or 500ml Histoplex jar lined with a 126µm nylon mesh paint strainer (Orr et al. 2003). Samples will be taken to the lab and frozen at -20°C within 6 hours of collection (King et al. 2008). Later, samples will be thawed and filled with ethanol before being manually homogenized with a disposable depressor inside the paint strainer to separate the scat matrix material from hard prey remains (e.g. bones, cephalopod beaks). The paint strainer containing prey hardparts will then be removed from the jar leaving behind the ethanol preserved scat matrix for genetic analysis (Thomas et al. 2014).

Prey hardparts Analysis

To remain consistent with the way previous harbor seal diet work in the region has been conducted using hard prey remains (i.e. hardparts), we will use the “all structures” approach to identify harbor seal prey contained in individual scat samples. Prey hardparts retained in the paint strainers will be cleaned of debris using either a washing machine or nested sieves. All diagnostic prey hardparts will be identified to the lowest possible taxon using a dissecting microscope and reference fish bones from Washington and British Columbia, in addition to published keys for fish bones and cephalopod beaks. Samples containing prey hardparts identifiable only to the family level (e.g. Clupeidae) and bones identifiable to the species level of the same family (e.g. Pacific herring) will both be tallied (Lance et al. 2001).

Salmonid bones recovered from seal scats will be differentiated into either adult or juvenile based on visual inspection by a morphological prey identification expert. A clear size difference exists between juvenile and adult salmon bones that is apparent to taxonomists upon visual inspection (Figure 2).

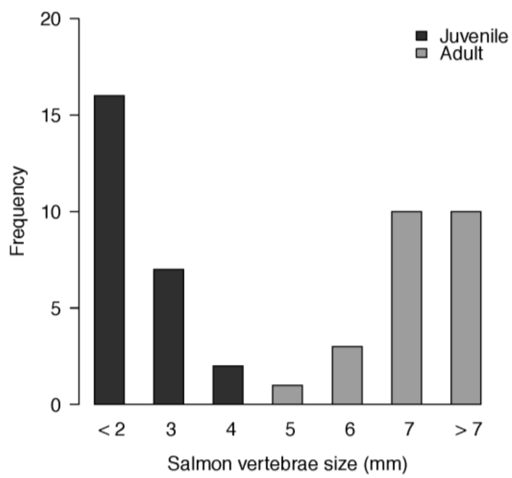

Figure 2. (From Thomas 2015) Frequency of salmon vertebrae between <2 mm and >7 mm, demonstrating the size difference between adult and juvenile salmon bones in seal scats.

DNA metabarcoding diet analysis

The DNA metabarcoding marker we will use to quantify fish proportions is a 16S mDNA fragment (~ 260 bp) previously described in Deagle et al. 2009 for pinniped scat analysis (Deagle et al. 2009). We will use the combined Chord/Ceph primer sets: Chord_16S_F (GATCGAGAAGACCCTRTGGAGCT), Chord_16S_R (GGATTGCGCTGTTATCCCT), Ceph_16S_F (GACGAGAAGACCCTAWTGAGCT), and Ceph_16S_R (AAATTACGCTGTTATCCCT). This multiplex PCR reaction is designed to amplify both chordate and cephalopod prey species DNA.

To ensure accurate salmon species identification, a secondary metabarcoding marker will be used to quantity the salmon portion of seal diet, because the primary 16S marker is unable to differentiate between coho (Oncorhynchus kisutch) and steelhead (Oncorhynchus mykiss) DNA sequences. This marker is a COI “minibarcode” specifically for salmonids within the standard COI barcoding region: Sal_COI_F (CTCTATTTAGTATTTGGTGCCTGAG), Sal_COI_R (GAGTCAGAAGCTTATGTTRTTTATTCG). The COI amplicons will be sequenced alongside 16S such that the overall salmonid fraction of the diet will be quantified by 16S, and the salmon species proportions within that fraction will be quantified by COI.

For all DNA sequences successfully assigned to a sample, a BLAST search will be done against a custom 16S or COI reference database. A sequence will be assigned to a species based on the best match in the database (threshold BLASTN e-value < 1e-20 and a minimum identity of 0.9), and the proportions of each species’ sequences will be quantified by individual sample after excluding harbor seal sequences or any identified contaminants (Caporaso et al. 2010). Samples will be excluded from subsequent analysis if they contain < 10 identified prey DNA sequences.

Harbor seal population diet percentages will be calculated from the DNA sequence percentages of individual samples in a collection - where seal population diet percentage for a particular prey species represents the average species DNA sequences % calculated from all samples in the collection. The percentage of juvenile steelhead in harbor seal population diet will be estimated based on the co-occurrence of steelhead DNA and juvenile salmon bones in seal scat samples (Thomas 2015).

DNA diet data will be provided in tabular form including: site, sample collection date, species, percent of diet, sample count, etc.

Pending identifying bioenergetics support, collaborators at WDFW and NMFS will use the resulting percentage of juvenile salmonid and steelhead in harbor seal diet (combined with seal population size and energy requirements) to estimate the numbers of juvenile steelhead eaten by seals in South Puget Sound. Lastly, comparisons will be made between the seal-related steelhead mortality rate (based on scatological analysis) and the survival of steelhead through South Puget Sound. Scat-based estimates of steelhead mortality from seals will be compared to telemetry-based estimates of predation as a means of validation for both methods.

Outcomes

The principle product of this work attempts to directly assess seal predation on juvenile steelhead and salmon in Puget Sound. The work will provide an estimate of seal population diet comprised of juvenile steelhead and salmon. The data may also be used to estimate the numbers of juvenile steelhead each in South Puget Sound, pending bioenergetics support. Further, with all fish species (salmonid and non-salmonid) and their percentages of diet included in the diet estimate, these data will provide a basis for identifying changes in the harbor seal diet in relationship to changes in prey presence and abundance (e.g., steelhead, hatchery salmon, forage fish). The data produced by this work will be used to compare estimates of seal predation among years 2016-2018 and to previous years captured in previous studies (Lance and Jeffries 2009).

Timeline

Activity	Start Date
Field work logistics	January 2018

Collection of seal scat samples	March – June 2018
Sample processing (hardparts)	July – November 2018
Sample processing (DNA metabarcoding)	July – November 2018
Data analysis (Bioinformatics + Diet %)	December – March 2019
(Pending) Data analysis (Bioenergetics – NMFS)	December – May 2019
Reporting	June 2019

Deliverables

The deliverables from this study will include presentations of the study findings to interested parties and at relevant scientific meetings. In addition, the data products from this study will be incorporated into one or more scientific publications assessing the impact of harbor seals on salmon and steelhead in Puget Sound. At a minimum, the manuscript will be a straightforward description of the seal diet over the steelhead migration period. If agreed upon by collaborators of the overarching Salish Sea Marine Survival Project, the final manuscript will publish all of the 2016-2018 diet data collected as part of this project, along with 2011-2017 diet data from the Strait of Georgia, in an open access data journal. The report will provide the data in a transparent fashion and fully articulate all of the methods, limitations, biases and assumptions.

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Study 5: Relating steelhead characteristics and environmental variables with steelhead smolt survival in Puget Sound and total marine survival trends

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Summary

We need to understand what variables are most related to steelhead smolt survival within Puget Sound and the rest of their marine stage in order to design the best management strategies for these fish. Specifically, environmental conditions faced by steelhead in both their freshwater and marine environments likely influence, either directly or indirectly, smolt survival in Puget Sound and the North Pacific Ocean. Freshwater processes may influence smolt survival trends, driving fish to be weaker as they enter the marine environment. Factors and conditions in Puget Sound and the larger marine environment may also limit steelhead abundance and productivity. For example, harbor seals may be consuming steelhead and contributing to their low marine survival rates. However, this predation may be mediated by forage fishes' availability (acting as buffer prey) along with other environmental variables, and it's important to assess these relationships over time. Additionally, by evaluating which individual fish and population traits are most correlated with survival rates over time, and specifically which characteristics are related to higher values, we can identify which are important to maintaining healthy populations. Factors that influence the balance of the life history portfolios across and within populations are considered crucial to recovery (Carlson and Satterthwaite 2011, McPhee et al. 2007).

Objectives

We will analyze existing data on wild and hatchery steelhead individual and population characteristics and environmental data and relate these variables to steelhead smolt-to-adult survival (SAR) trends and steelhead smolt survival rates specifically in Puget Sound (derived with acoustic telemetry work) over time. Environmental data will be collected at three spatial scales and include variables such as (but not limited to) river flow, temperature, salinity, turbidity, productivity, upwelling, predators, and buffer prey. The goal of this work is to evaluate the relationship between SAR and Puget Sound survival differences and 1) variation in population life-history diversity and 2) the physical environment of steelhead and conditions therein. We will examine hypotheses related to spatial variation in mortality, size-selective mortality, predator-prey interactions, foraging arena theory, match-mismatch, and life history variation. We will address the following questions:

1. Are SAR and Puget Sound survival data correlated with changes in hatchery and wild steelhead and salmon smolt abundance?

We seek to understand the timing and scale of hatchery smolt contribution to the pelagic biomass for Puget Sound, coastal Washington, and lower Columbia River areas. We hypothesize that SAR and Puget

Sound survival data are correlated with changes in hatchery and wild steelhead and salmon smolt abundance and entry timing. A positive correlation (e.g., higher survival rates when higher smolt abundance) would indicate a predator-swamping effect, where more smolts mean that predators are satiated and thus consume a lower fraction of the population. Alternatively, a negative correlation would indicate density-dependent effects, where more smolts mean fewer resources and thus lower survival for the average individual. Furthermore, higher survival rates relative to lower smolt abundance may indicate predator disinterest.

2. Are SAR and Puget Sound survival trends correlated with population life-history diversity?

We hypothesize that SAR and Puget Sound survival trends are correlated with population life-history diversity and that populations that have declining diversity will have lower survival rates.

3. Which ecosystem indicators best predict steelhead early and total marine survival?

The goal of this work would be to understand whether any ecosystem indicators predict steelhead marine survival and thus which may be most associated with changes in Puget Sound steelhead abundance and viability. We would also evaluate whether different environmental factors are more strongly correlated with marine survival variables in different regions of Puget Sound to examine hypotheses concerning spatial variation in mortality.

a. Buffer prey abundance?

Do changes to pelagic fish (or squid) biomass correlate with changes to steelhead early marine survival? If there is a buffer prey (or conversely, predator attraction) effect, is it primarily driven by abundance, species composition, distribution or a combination thereof? We hypothesize that, in years when and where more buffer prey species (such as forage fishes) were present, steelhead survive at a higher rate in Puget Sound. We will focus on data describing offshore biomass of these fishes, including herring and anchovy, to the best extent practicable. We will consider abundance, individual size, and spatial and depth distribution (where data are available) of potential buffer prey during the steelhead outmigration period (April-June). We will compare our findings to known changes in Puget Sound seal diet composition in different years where Puget Sound steelhead smolt survival rates have differed. Finally, we will analyze potential differences between steelhead mortality in Hood Canal and south and central Puget Sound.

b. Predator abundance?

This includes prey for buffer prey (e.g., zooplankton) along with predator abundance or distribution (e.g., harbor seals, harbor porpoise, cormorants and other piscivorous birds). We will also assess if predator disturbances (e.g., activity of transient whales) correlate with higher early marine survival (and/or SARs) of steelhead. If so, what disturbance results in this effect, and where?

Study Design

SAR data for Puget Sound, coastal Washington and Oregon, lower Columbia River, and the Keogh River of BC, Canada will be statistically analyzed and correlated with the variables listed above. We will also perform statistical analyses using Puget Sound-specific survival data from acoustically-tagged fish. Such analysis will help determine whether certain characteristics or conditions are contributing uniquely to mortality (or are uniquely affected by the environment).

The following fish characteristics will be included in this analysis, when data are available: hatchery broodstock type (e.g., Chambers, Skamania, native); broodstock management approach used for hatchery programs (integrated vs. segregated); hatchery/wild composition and introgression in natural-origin populations; wild smolt outmigration timing; hatchery smolt release timing; and hatchery and wild smolt counts across salmonid species. We will quantify hatchery salmon and steelhead biomass input, along with their specific entry dates, in May to June (when steelhead smolts enter).

Environmental data will be collated at three spatial scales as steelhead from the different regions first encounter different environments but then all spend time together in the Pacific Ocean: watershed specific, Puget Sound basin specific, regional, and ocean-wide. Variables that will be estimated at each scale, when possible, include (but are not limited to) those related to river flow, temperature, salinity, turbidity, productivity, dissolved oxygen, upwelling, large-scale oceanographic indices, predator abundance, and buffer prey.

For example, we will ask whether variation in body size, migration timing, or life-history characters affect marine survival. Such analyses will help determine whether certain characteristics are contributing uniquely to mortality (or are uniquely affected by the environment) in Puget Sound. Specifically, disparity between the marine survival performance of populations released/that outmigrate in the summer or fall compared to those that outmigrate in the spring may help indicate whether food supply is an issue and the extent to which the spring bloom is playing a primary role.

We will also evaluate whether decreases or increases in salmonid smolt abundance may be affecting predator-prey interactions (high abundance resulting in buffering or low abundance resulting in predators ignoring steelhead) or whether high abundances could be correlated with density-dependent effects. This evaluation will be performed at the watershed, sub-region (south Puget Sound, central Puget Sound, north Puget Sound, Hood Canal, Strait of Juan de Fuca), and Puget Sound region levels.

For data for which long-term trend data are available, we will employ statistical methods including regression models (to understand which factors best predict the survival rates in Puget Sound and SARs), time series methods (to test for the presence of variations and patterns over time), and correlations (to examine relationships between Puget Sound survival and SAR trends and the predictor variables). Specifically, we will evaluate the usefulness of multiple methods including dynamic factor analysis, principle component analysis, and state-space models. Mixed effects models will also be incorporated where needed, where the random effect (with multiple samples for a given sampling object) is watershed, sub-region, or year. For some variables, though, long term data are not available and only snapshots of certain conditions exist. In these cases, more qualitative analyses will be used to examine relationships between these variables and the Puget Sound survival and SARs.

Nisqually River steelhead early marine survival data was estimated for 2006-2009, and 2014-2017 using acoustic telemetry, with plans to measure survival in 2018 and 2019 as well. Hypothesis-driven analysis will be used to determine which of a few hypothesized factors explain the variability in the survival data. Potential indicator variables include (1) transient killer whale presence in Puget Sound, which may positively affect survival estimates if their prey (i.e., harbor seals, harbor porpoise) numbers or behavior are altered; (2) Pacific Decadal Occilation (PDO) or Puget Sound temperature, which may influence the recruitment of forage fish, specifically Northern anchovy, and potentially buffer steelhead predation in Puget Sound during years of high recruitment; and (3) predator abundance, which may have a direct effect on steelhead survival. Linear models will be compared and examined to determine the most likely factors influencing survival.

Timeline

We have collected SAR data from populations in western Washington and Oregon from the late-1970s to present. We have performed QA/QC methods to determine the best dataset to use in the analyses described here. Much of the fish characteristics and environmental data have already been collated in prior biennia but work on this front will continue, with additional data being gathered from Washington Department of Ecology, University of Washington, WDFW, and others. The modeling will continue in 2017 and will be completed in 2018. This work will be submitted to a peer-reviewed publication by August 2018. Analysis of early marine survival data has begun informally as indicator datasets are developed, but will start more formally when 2019 survival data are available from OTN (December 2019). The results of that analysis will be written up and submitted by summer of 2020.

Deliverables

The deliverables from this study will be at least two peer-reviewed manuscripts and presentations of the study findings to interested parties and at relevant scientific meetings. The datasets collected during this project will be used for analyses of factors related to marine survival rates of Pacific salmon in Puget Sound.

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