SALISH SEA
MARINE SURVIVAL PROJECT

Puget Sound Steelhead Marine Survival: 2015-2017 Research Work Plan

Puget Sound Steelhead Marine Survival Workgroup

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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 (WDFW) and Puget Sound Partnership (PSP) initiated an effort to determine why steelhead are dying in Puget Sound. Given the level of uncertainty regarding the factors affecting steelhead early marine survival, a multi-disciplinary, ecosystem-based research approach was chosen. To achieve this, the Puget Sound Steelhead Marine Survival Workgroup (Workgroup)[1] was formed, including experts from state and federal agencies, Puget Sound Treaty Tribes, and academic representatives. This Workgroup is coordinated by the nonprofit, Long Live the Kings, and is a component of the Salish Sea Marine Survival Project[2].

The initial research phase was funded by a Washington State appropriation of $788,000 (2013-2015 biennium) via PSP and $800,000 of direct match in equipment, services, and staff time from collaborators. Through ten studies implemented in the initial research phase (2013-2015), the Workgroup determined that the causes of mortality are most likely derived in the lower-river or marine environments, and b) predation and disease are likely the most significant factors affecting survival. However, how these factors interact, the degree to which these factors are affecting survival varies among Puget Sound steelhead populations, and environmental characteristics that may exacerbate these factors must be understood. Also, other factors may be contributing to this mortality, at least for some populations, and should be investigated further. Please see Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2015 research findings summary[3] for more information.

A second Washington State appropriation of $800,000 was provided in 2015-2017 to WDFW to support the next steelhead marine survival research phase. Significant match is again being provided from the research collaborators. The next phase of research focuses on determining the extent of mortality occurring from each source, how the sources of mortality interact, and the specific ecosystem dynamics that have changed over the past 30 years and led to this mortality.

[1] Puget Sound Steelhead Marine Survival Workgroup members are listed on the back of the cover of this report.

[2] The Salish Sea Marine Survival Project is a US-Canada research initiative to determine the primary factors affecting juvenile chinook, coho, and steelhead survival in the combined marine waters of Puget Sound and Strait of Georgia. Visit www.marinesurvivalproject.com for more information.

[3] Puget Sound Steelhead Marine Survival Workgroup. September 2015. Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2015 research findings summary. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com.

Specifically, the list of studies includes:

- Quantifying the impact of harbor seals on wild Nisqually River steelhead through two complimentary methods: acoustic telemetry and seal scat analysis. The Nisqually River acts as a sentinel stock given that it must traverse much of Puget Sound, has high early marine mortality rates, and has multiple years of data, including accurate accounts of outmigrant abundance, to build upon.
- Determining the effects of the parasite, *Nanophyetus*, on swimming performance and survival of Puget Sound steelhead smolts via an experimental approach. In 2013-15, the Workgroup found that steelhead from in the Green and Nisqually rivers had high levels of *Nanophyetus* once they reached the lower river and Puget Sound marine environment. Two groups of steelhead, one infected with *Nanophyetus* and one uninfected, will be acoustic tagged and released into Puget Sound. Their survival rates will then be compared.
- Pending an internal US Geological Survey funding allocation, USGS staff will finish developing and then utilize an eDNA tool to determine the locations and timing of *Nanophyetus* hotspots in Puget Sound rivers. If the Workgroup finds that Nanophyetus is indeed contributing to higher mortality rates, these data can be used to determine areas to treat for *Nanophyetus*, or to avoid altogether.
- Work will continue on assessing correlations between steelhead biological characteristics and/or environmental variables with Puget Sound steelhead marine survival trends.
- If funded, Genome-wide association studies (GWAS) of survival and prevalence of pathogens and pathologies in outmigrating steelhead will be performed. The work builds upon a preliminary analysis performed in 2013-15 that showed that there may be an association between the expression of specific genes and mortality in Puget Sound. It would also test whether there is an genomic association with the intensity of *Nanophyetus* infections, infections from other diseases found, and their symptoms.
- Coho from South Sound Net Pens will be acoustic tagged and tracked via a complementary effort by the Squaxin Tribe. Their intent is to determine whether similar mortality sources and patterns are occurring for coho as steelhead, taking advantage of the acoustic tag tracking that will be implemented for steelhead work in 2016. This information may also shed light on whether the pulse abundance of hatchery coho are attracting predators to South Puget Sound, increasing the mortality of coho and co-migrating steelhead that may otherwise survive better in more dispersed outmigration groups.
- Additional analyses of lipid contents will be performed to compare to the intensity of the *Nanophyetus* parasite in individual fish, to see if they are correlated. Secondarily, chemical analyses of juvenile steelhead collected in 2015 at the Nisqually River smolt trap will be repeated (as was done in 2014) to determine if high PBDE contamination levels in juvenile steelhead are a consistent problem in this river system. These PBDE results will then be used to determine if additional PBDE studies are needed in 2016.

Over the next two years, members of the Workgroup will also work with the Puget Sound Steelhead Recovery Team and the Salish Sea Marine Survival Project Coordinating Committee to create the foundation for converting the results of this research effort into recovery actions.

2015-2017 RESEARCH

From January through September 2016, the Workgroup reviewed their findings from the first phase of research, discussed relevant objectives for the next phase, and developed and prioritized studies to address the objectives. This section describes the framework, objectives and proposed studies for the 2015-2017 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[4] 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)[5], and the 2013-2015 findings summary[6]. This information provides the basis for the Workgroup's objectives and affiliated research for 2015-2017 work phase.

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

[5] 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.

[6] Puget Sound Steelhead Marine Survival Workgroup. September 2015. Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2015 research findings summary. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com.

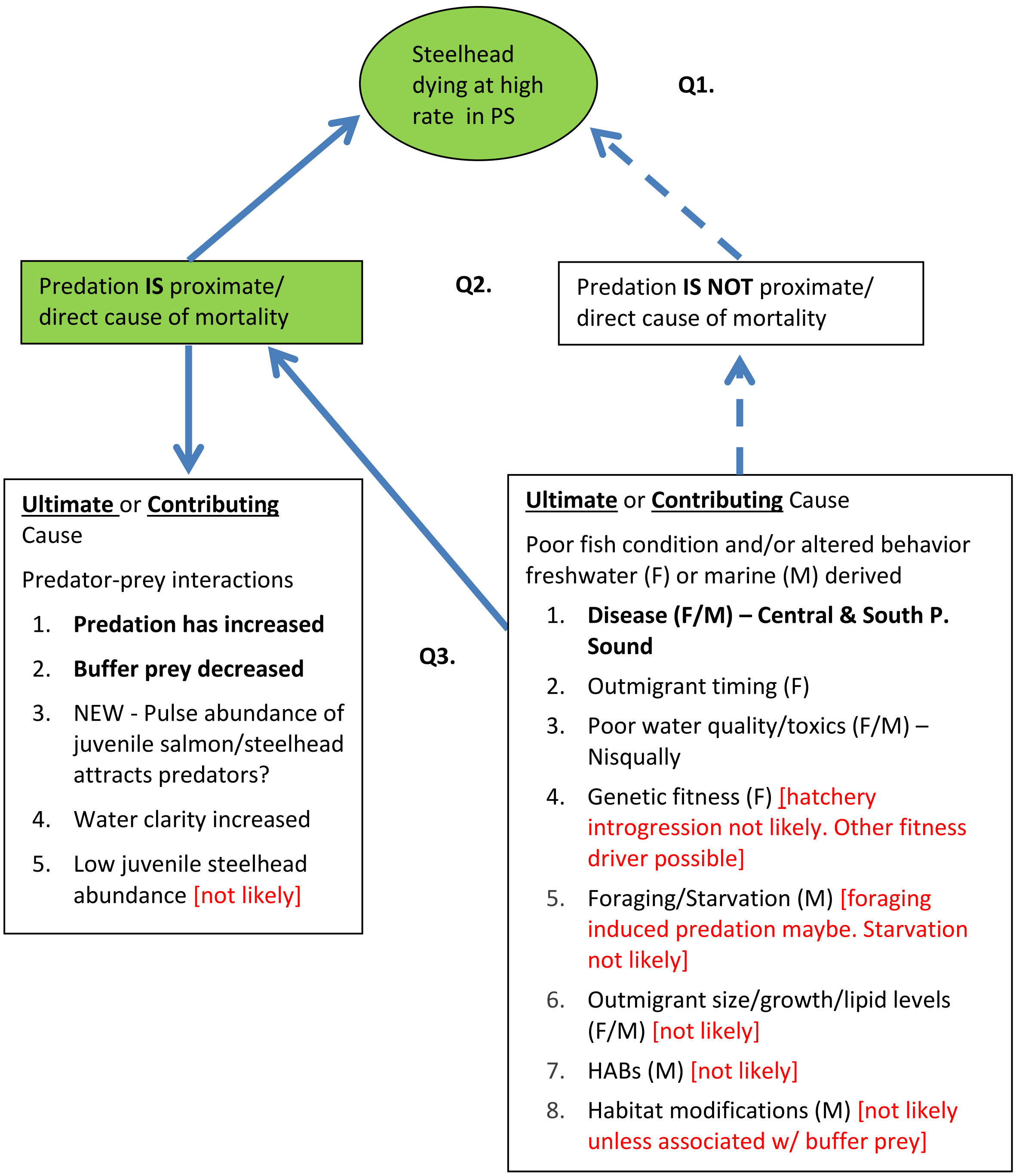


Figure 1. Updated Puget Sound steelhead marine survival logic model. The factors are ranked based upon existing evidence. Q1, 2, and 3 refer back to the three-question framework of the research effort.

Objectives

The following objectives were established by the Workgroup for the 2015-2017 research phase. These objectives represent the next steps recommended by the Workgroup, based upon the evidence collected to date, described in short-hand in the logic model of the research framework, above. The objectives guided study development and prioritization. The objectives in red font were discussed but not directly addressed in the suite of proposed studies for 2015-2017. See the section titled, "Deemed not feasible, not the appropriate time, or to be dealt with via other processes" in Table 1 of Appendix B for discussion details and recommended next steps for objectives C, G and Q. The Workgroup is also investigating approaches for combining the early marine survival data with contemporary environmental data to better investigate correlations (objective F).

Predator-prey interactions and environmental drivers

A. Continue to focus the research on one of the most likely predator(s) (harbor seals).

B. Estimate a predation rate by harbor seals on steelhead in Puget Sound (south of Admiralty Inlet), and determine whether predation by harbor seals differs by region.

C. Secondarily, assess predation by other potential predators, in particular harbor porpoises, double-breasted cormorants, and Caspian terns.

D. Increase the power of the study to determine whether the sound emitted by the acoustic tags used in early marine survival research attracts auditory predators and results in a bias to the mortality rates observed (aka. the dinner bell effect).

E. Complete the retrospective examination of potential environmental influences of predator-prey dynamics and assess linkages in detail (predator abundance, hatchery coho abundance, dissolved oxygen, water clarity, etc).

F. Use contemporary telemetry data describing early marine survival to further investigate linkages with environmental influences that stand out in the retrospective examination. Look within years as well as among years.

G. Determine whether there is a relationship between food availability, outmigration rate/behavior, and exposure to predation.

Poor fish condition and or altered behavior

H. Determine whether new infections of *Nanophyetus* cause direct, disease-related mortality or indirect mortality by compromising the physical condition of migration steelhead smolts, thereby predisposing them to a greater risk of predation.

I. Assess temporal windows of *Nanophyetus* cercarria shedding events, and spatially, what zones in watersheds are most infected with *Nanophyetus*.

J. Increase the power of GWAS investigation to better determine whether smolts in some populations with specific genetic fingerprints may be compromised by their morphology (fin development) or immunological responses, making them sick or more vulnerable to predation.

K. Determine whether there is a relationship between the GWAS results and *Nanophyetus* loads OR pathology consistent compromised health resulting from infections (inflamed heart and gills).

L. Investigate PBDE inputs in the Nisqually River. Again assess outmigrating steelhead to determine if PBDE loads are a consistent problem.

M. Better assess whole body lipid content and condition factor relative to *Nanophyetus* loads and survival of outmigrating steelhead trout.

N. Complete retrospective examinations of hypotheses concerning spatial variation in mortality, size-selective mortality, match-mismatch, life history variation.

Apply to both of the above

O. Assess why juvenile steelhead migrating in April and late May survive at higher rates than steelhead migrating in early-mid May. Primarily, investigate whether this may be associated with factors such as changes in predator-prey dynamics (prey switching driven by pulse abundance of hatchery coho) or *Nanophyetus* shedding events/disease outbreaks. Secondarily, assess other potential correlates in existing data, such as changes in water clarity, food availability, and harmful algae blooms.

P. Continue to utilize the Nisqually steelhead population because it epitomizes marine survival issues and because the population is well tracked. The Nisqually River has a relatively high amount of habitat protection, no hatchery steelhead releases, large smolts and one of the best smolt abundance estimates for Puget Sound populations.

Q. Use modeling to combine drivers and build out to broader ecosystem effects.

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. The studies were then prioritized by the entire Workgroup regarding:

- The relative importance of each study or study component as next steps toward determining the primary factors affecting the early marine survival of juvenile steelhead.
- The likelihood of success at achieving the Work Plan objectives affiliated with each activity.

The prioritization exercise was used to high-grade proposals within the funding available, but did not result in any particular study being eliminated from the Work Plan. See “Appendix B: Information Supporting Workgroup Decision Making” for detailed information regarding each study/research component, considerations affiliated with each, and the resulting study ranking.

The studies are listed in order of priority based on the Workgroup ranking. The studies also cross-reference the objectives they address, listed in the previous section. The funding status for each is described. See “Appendix A: Study Descriptions” for complete descriptions of each study. For more information about the current state of funding, see the “Budget and Funding Strategy” section of this report.

Study 1: Quantifying juvenile steelhead in harbor seal diet using scat analysis

Affiliated objectives = A, B, P

It is currently hypothesized that harbor seal predation is a mechanism responsible for low juvenile steelhead survival during outmigration through Puget Sound. Acoustic telemetry work conducted in Puget Sound and Hood Canal has produced indirect evidence suggesting that harbor seals are consuming outmigrating juvenile steelhead in the marine environment, but we currently lack direct evidence of steelhead predation by harbor seals during the critical smolt outmigration time period. This study aims to provide direct evidence by identifying and quantifying steelhead DNA in fecal samples of Puget Sound harbor seals collected during the outmigration window. The percentage of juvenile steelhead in the Puget Sound harbor seal diet will be estimated based on the co-occurrence of steelhead DNA and juvenile salmon bones in seal scat samples. These data, combined with quantification of all other prey species, will yield a percentage of seal global diet comprised of juvenile steelhead. Those percentages can then be merged with seal bioenergetic data and a population census to estimate the biomass of juvenile steelhead (or number of individuals) consumed by harbor seals in Puget Sound. The advantage of this approach is that it eliminates doubt regarding the proximate source of mortality for juvenile steelhead, and serves as a complimentary validation for telemetry-based seal predation estimates.

Status = Funded. An assessment of South Puget Sound seal diets was funded. The focus on South Puget Sound was due to cost limitations and the ability to quantify the juvenile steelhead outmigrant abundance in South Puget Sound, required to establish a consumption estimate. South Puget Sound is dominated by Nisqually steelhead. Nisqually steelhead outmigrants are counted each year by WDFW at a smolt trap on the Nisqually River. If additional funds are obtained, this study may be expanded to include the Central Puget Sound and Admiralty Inlet regions.

Study 2: Interactions between harbor seals and steelhead in Puget Sound

Affiliated objectives = A, B, D, H, O, P

Studies conducted in 2014 provided the first information on temporal and spatial overlap between harbor seals and steelhead trout in Puget Sound, including inferred harbor seal consumption of steelhead smolts. Harbor seals outfitted with GPS tags and acoustic transceivers detected steelhead implanted with acoustic telemetry transmitters (tags) both during and after the smolt outmigration period. Together with data from fixed arrays, we were able to categorize the fate of approximately one third of the steelhead entering Puget Sound as either having survived to Admiralty Inlet, stationary at a haulout location, or still present in Puget Sound (and presumed stationary) after the smolt outmigration window. Steelhead smolts tagged with silent tags had very similar odds of survival through Puget Sound than smolts tagged with continuously pinging tags, providing no evidence that tag noise affected the survival of steelhead migrating through Puget Sound. However, the Workgroup has initially concluded that the power of this study component—to determine whether there is a potential for a “dinner bell effect”—should be increased to a point where the sample sizes are large enough to detect an absolute difference in survival of 10% to detections at Admiralty Inlet and Strait of Juan de Fuca acoustic receiver arrays. The recommended sample size to obtain this resolution (n=250) was based upon 2014 detections at the acoustic arrays. The sample size needed may be reduced by the increased power to detect tags in 2016; therefore, the Workgroup will continue to analyze and build upon annual results until the power issue is satisfied.

The primary objectives of this study are to estimate a predation rate by harbor seals on steelhead in Puget Sound (south of Admiralty Inlet), and determine whether predation by harbor seals differs by region. 200-250 Nisqually river steelhead will be acoustic tagged, and 12- 18 seals will fitted with GPS/acoustic receiver instrument packs. Tag detection capabilities will be expanded by: i) monitoring harbor seals in South and Central Puget Sound and Admiralty Inlet, ii) placing stationary receivers at seal haulouts and at random locations not frequented by harbor seals, and iii) conducting mobile tracking to locate tags remaining in Puget Sound after the smolt outmigration period. Seal time at depth and locations will be quantified in such a manner that estimates the amount of time seals spend at haulout locations. This information will be used to estimate the probability that a tag consumed by a harbor seal would be defecated near a haulout site. Data on harbor seal abundance, behavior, steelhead tag locations, and smolt abundance will be combined to estimate the predation rate and total number of smolts consumed by harbor seals.

Finally, a sub-sample of steelhead (n=50 to 100) will be implanted with delayed pinging tags that remain silent through Central Puget Sound, then activate in Admiralty Inlet, to continue to test the potential for a dinner bell effect. If funding becomes available, Nisqually steelhead will again be tagged in 2016 and the results over years 2014, 2016, and 2017 will be compiled.

Status = Funded. Current funding limitations only allow for the purchase of 12 instrument packs (to tag 6 seals in the Nisqually estuary/Gertrude Island area and 6 seals at Orchard Rocks) and the tagging of 50 steelhead with delayed pinging tags (200 steelhead tagged, total). If additional funds are obtained, this study may be expanded to include an additional 6 harbor seals, tagged at Colvos Rocks, and 100 delayed pinging tags.

Study 3: Spring assessment of harbor seal numbers and distribution in Puget Sound

Affiliated objectives = A, B

Aerial surveys will occur to estimate the number and distribution of seals in Puget Sound between Point Wilson and South Puget Sound. Flights will occur in April, May and June around daily low tides targeting a surveys window between two (2) hours before low tide to two (2) hours after low tide, when maximum numbers of harbor seal are known to haul out. All known haulout sites will be surveyed, and any new haulout sites will be identified during each flight. Seals or sea lions in the water will be recorded when encountered. Data collected during surveys will include date, time, and location, as well as a visual best estimate of seal numbers. Photographs will be collected of all sites where more than twenty-five (25) seals are hauled out. The digital images from the aerial photos will be counted to record total seal (and pups) and sea lion numbers for each site. Methodologies will be consistent with Jeffries et al. (2002). Counts will be entered into the WDFW seal and sea lion aerial survey database.

Study 4: Effects of *Nanophyetus* on swimming performance and survival of steelhead smolts

Affiliated objectives = H

Recent fish health assays indicate high infection prevalence (87-100%) and intensity (800-2500 cysts/fish) of *Nanophyetus salmonica* in steelhead outmigrating from Central (Green) and South (Nisqually) Puget Sound Rivers. South and Central Puget Sound steelhead populations generally experience lower early marine survival rates than those from North Puget Sound rivers, where *Nanophyetus* infections in assayed steelhead were absent (Chen et al. in preparation). Steelhead are exposed to *Nanophetus* as outmigrating juveniles after an infectious stage of the parasite is shed into the water column from the intermediate snail host. The extent to which the *Nanophyetus* infections contribute to high observed mortality rates during the early marine phase of the juvenile steelhead life history remains unclear. High infection intensity among freshwater outmigrants could contribute to rapid mortality shortly after seawater entry or to reduced swimming performance. A direct survival comparison of infected and healthy steelhead is needed to quantify and assess the effects of *Nanophyetus* infection on steelhead survival.

This study will use acoustic telemetry (assisted by the infrastructure described in Study 2) to evaluate differences in the early marine survival (near river mouths to the Pacific Ocean) of pathogen-free (SPF) and *Nanophyetus*-infected Puget Sound steelhead smolts. This approach will help us understand effects of *Nanophyetus* infection at different stages of the steelhead smolt migration through Puget Sound. The study will also test whether *Nanophyetus* infections affect swimming ability and survival after seawater transfer.

Status = Funded. Current funding limitations only allow for 300 acoustic tagged steelhead (150 treatment, 150 control), whereas the original proposal called for 400 acoustic tagged steelhead. The smaller sample sizes slightly reduce the ability to detect differences in *Nanophyetus*-infected vs non-infected steelhead. If additional funds are obtained, this study may be expanded to include 400 as originally proposed.

Study 5: Toward management of *Nanophyetus* - Assessing *Nanophyetus* exposure zones

Affiliated objectives = I

This study involves some forward thinking and its results will be most pertinent if we find that *Nanophyetus salmincola* do contribute to higher early marine mortality rates via Study 4, above. Prior to recommending or implementing disease management strategies, a better ecological understanding of *Nanophyetus* is needed, particularly regarding the factors that influence infection pressures in endemic watersheds. Using an eDNA approach—a quantitative polymerase chain reaction (qPCR)—t targeted against specific regions of the *Nanophyetus* genome is currently being developed. Once developed and optimized, this tool will be used to:

- Assess the diurnal, seasonal, and longitudinal details of *Nanophyetus* shedding in endemic watersheds.
- Assess the geographical and watershed-wide distributions of *Nanophyetus*-positive *Juga* spp. snails throughout the region.

By detecting *N. salmioncola* in water and snail samples at different times and locations throughout the watersheds, we will identify:

- Temporal windows of opportunity when out-migrating steelhead might avoid *Nanophyetus* exposures.

- Zones in the watershed that could be treated to remove infected snails or free-swimming cercarria.
- Spatial infectious zones in affected watersheds that should be avoided by steelhead via transport or exclusion.

Status = Pending internal allocation of USGS funds

Study 6: Relate steelhead characteristics and environmental variables with smolt marine survival trends

Affiliated objectives = E, N

The goals of this work will be to evaluate whether changes in population life-history diversity, juvenile salmonid density, and environmental conditions at multiple spatial scales are associated with variable steelhead smolt-to-adult return (SAR) rates. We will examine hypotheses concerning spatial variation in mortality, size-selective mortality, match-mismatch, and life history variation. Specifically, steelhead individual and population characteristics along with environmental variables will be related to western Washington and Oregon smolt marine survival trends. Fish characteristics include hatchery characteristics, smolt size, smolt outmigration or release timing, and smolt counts across salmonid species. Environmental data will be collated at three spatial scales (watershed specific, Puget Sound basin specific, regional, and ocean-wide) as steelhead from the different regions first encounter different environments but then all migrate through the Pacific Ocean. Such variables include those related to river flow, temperature, salinity, turbidity, productivity, dissolved oxygen, upwelling, large-scale oceanographic indices, predators, and buffer prey. We will employ quantitative, time-series models when long-term data are available and more qualitative assessments when only short-term data are available.

Status = Funded

Study 7: Genome-wide association studies (GWAS) of survival and prevalence of pathogens and pathologies in steelhead smolts outmigrating through Puget Sound

Affiliated objectives = J, K

This study effectively continues two projects completed in the 2013-2015 study phase. First, the power of the original Genome-wide Association Study (GWAS) will be improved by adding 2014 reciprocal transplant samples not included in the original GWAS, and the 2015 acoustically-tagged samples from the Nisqually River. These additional samples will, presumably, increase statistical power to enable an appropriate test for the association between genomes and survival within a broadly defined population of outmigrating steelhead smolts. In addition, unlike the original GWAS where fate was defined absolutely as either successfully migrating through Puget Sound (survival) versus not being detected by any acoustic receiver (mortality), in this new project we will define fate incrementally, enabling the use of all samples with genomic sequences. Second, a genomic perspective to the initial study on the prevalence and load of *Nanophyetus salmincola* in outmigrating steelhead smolts will be added. As in the first component of this effort, we will conduct a GWAS on a set of samples from the initial disease prevalence study; however, in this second study instead of testing for an association with survival, we will test for genomic association with the prevalence of primarily *Nanophyetus*, and secondarily myxosporean species, *Sanguinicola* spp, and gill and heart pathologies.

Status = Partially Funded. The Workgroup ranked this study lower than the others listed above, and the funds available from the Washington State Appropriation were initially insufficient to include this study. However, additional funding has been obtained by Long Live the Kings that to pay for a portion of this work: to improve the power of the initial GWAS study.

Study 8: Hatchery Coho Telemetry Study

Affiliated objectives = O, P

Telemetry studies of steelhead (*Oncorhynchus mykiss*) smolts in the Puget Sound have indicated that approximately 80% of fish entering marine waters do not survive to the Pacific Ocean (Moore et al., in review). Telemetry data for coho (*Oncorhynchus kisutch*) in South Puget Sound also suggest high early marine mortality (unpublished S. Steltzner, Squaxin Tribe). The long-term declines in smolt-to-adult survival for Puget Sound steelhead (unpublished Kendall, WDFW) and coho (Zimmerman et al. 2015) have been similar, suggesting that there may be a common source of mortality. Analyses of survival patterns have revealed that outmigration timing may influence the survival success of steelhead smolts migrating from river mouth to the Pacific Ocean. For example, Moore et al. estimated low survival rates of juvenile steelhead migrating through Puget Sound during the first week of May for several Puget Sound populations during each of four study years (2006-2009) in relation to higher survival rates in late April and late May. In 2014, survival rates of smolts from the Nisqually River declined linearly with release date from late April to late May (Megan Moore, unpublished data). One possible factor that may be driving these temporal patterns is the release of large numbers of hatchery coho salmon smolts. Large numbers of prey moving through south Puget Sound together may be attracting predators to the foraging area (aggregation response, see Wood 1985), increasing the mortality of coho and co-migrating steelhead that may otherwise survive better in more dispersed outmigration groups.

The proposed study would generate data on migration timing, abundance patterns, and mortality distribution of hatchery coho salmon smolts throughout Puget Sound. With this information we would be able to assess peak coho migration timing and compare temporal and spatial mortality patterns of steelhead and coho smolts, allowing us to identify whether mortality increases with juvenile coho abundance in South Puget Sound, and whether similar mortality sources (i.e. harbor seals) are affecting both populations.

Status = Funded. The Workgroup ranked this study lower than the others listed above, and the funds available from the Washington State Appropriation were insufficient to include this study. However, the Squaxin Tribe has agreed to fund the tagging of 100 hatchery coho from South Sound Net Pens to determine whether coho are experiencing the same fate as steelhead. Therefore, if steelhead tagging can be done over the appropriate time period, we will also be able to achieve the objectives of Study 8.

Study 9: Lipid content analysis and next steps with contaminants

Affiliated objectives = L, M

The lipids of 60 individual steelhead samples collected in 2014 will be analyzed: % lipids as well as composition of lipid classes. The detailed lipid results will be compared to the intensity of the

Nanophyetus parasite in individual fish to see if they are correlated. Secondarily, chemical analyses of juvenile steelhead collected in 2015 at the Nisqually River smolt trap will be repeated (as was done in 2014) to determine if PBDE contamination is a consistent problem in this river system. These PBDE results will then be used to determine if additional PBDE studies are needed in 2016.

Status = Funded via WDFW toxic contaminants program. The Workgroup ranked this study lower than the others listed above, largely due to evidence that suggests that low lipid levels are consistent with a decline in whole body lipid content toward depletion during the smolt life-stage. Furthermore, the Workgroup already knew that the toxic contaminants program would be funding follow-up work to the initial finding of high PBDE levels, above thresholds that could affect fish health, in Nisqually River steelhead.

Additional studies considered

Additional studies were considered, but ultimately not included. See the section titled, “Deemed not feasible, not the appropriate time, or to be dealt with via other processes” and “Discussed but not part of priorities” in Table 1 of Appendix B for details.

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

Over the next two years, members of the Workgroup will work with the Puget Sound Steelhead Recovery Team (Recovery Team) and the Salish Sea Marine Survival Project Coordinating Committee (Coordinating Committee) to create the foundation for converting the results of this research effort into recovery actions. WDFW scientists Neala Kendall and Joe Anderson participate in both the Workgroup and Recovery Team. During the summer of 2015, the Recovery Team determined that a task team should be formed, including both Workgroup and Recovery Team members, to determine how the results of this research will be translated to actions in the forthcoming Puget Sound Steelhead Recovery Plan. In addition, the Coordinating Committee, that includes lead science representation from the Puget Sound region's federal, state, and tribal management agencies, will work with the Workgroup on developing and implementing the initial steps toward recovery, to be carried out by their respective agencies. As the results of this research may suggest considerations for marine mammal management[7], hatchery management, disease control, and/or forage fish recovery, we will 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.

[7] 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.

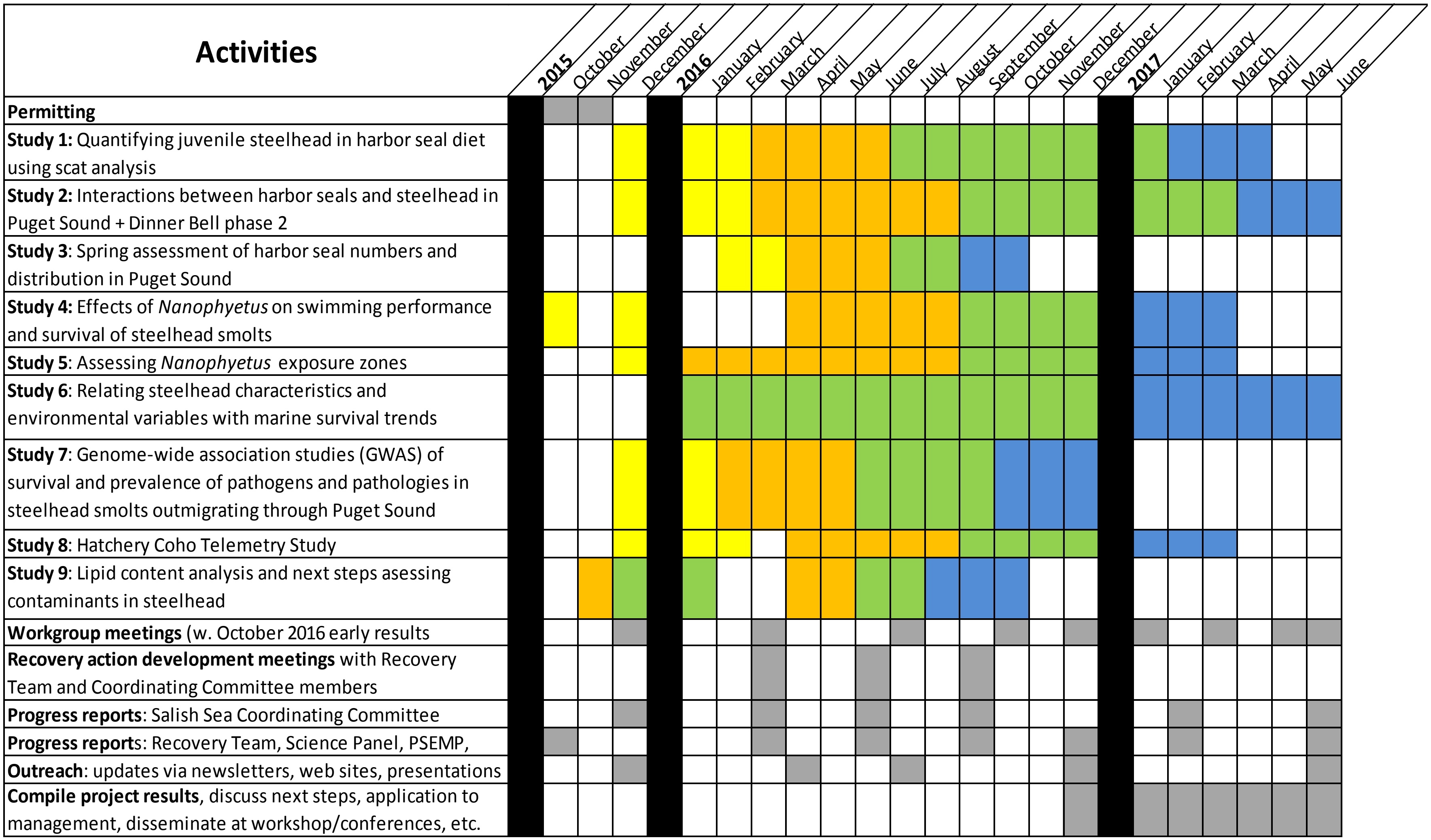
The results of all the studies in the work plan will be comprehensively evaluated by the Workgroup as a whole and will be presented to outside experts in aggregate for review and discussion. This will be led by the WDFW project manager, Neala Kendall, and project coordinator, Michael Schmidt of LLTK. A series of workshops 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 American Fisheries Society Conferences, the Salmon Ocean Ecology Workshop, 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. Study timeframes include: preparation, field work, analysis, reporting. All work is completed by June 30th, 2017.



BUDGET AND FUNDING STRATEGY

The following is a general budget for the 2015-2017 research phase. The total cost of the effort is between $1.4 and $1.7 million dollars. Approximately $600,000 of that is provided as match (wages and equipment) by the participating entities. Current revenue is being provided via at Washington State Appropriation and an activity cost share with the Nisqually Indian Tribe. Additional funds will be sought to implement the funded studies based upon their original scope, and to funded studies currently not supported by the existing funding sources. Funding decisions were made based upon research priorities. Funded studies were modified without significantly compromising their work, in order to maximize funds available. For additional details regarding the studies and how they were modified, see the "Overview of Studies" section, above.

2015-2017 Puget Sound Steelhead Marine Survival Budget	**Funded Expenses**	**Match**	**Additional Need**	**Study Total**
Study 1: Quantifying juvenile steelhead in harbor seal diet using scat analysis AND **Study 3:** Spring assessment of harbor seal numbers and distribution in Puget Sound	$140,341	$20,000	$55,715	$216,056
Study 2: Interactions between harbor seals and steelhead in Puget Sound + Dinner Bell phase 2	$404,552	$342,154	$106,347	$853,053
Study 4: Effects of *Nanophyetus* on swimming performance and survival of steelhead smolts	$117,353	$0	$26,956	$144,309
Study 5: Assessing *Nanophyetus* exposure zones	$0	$200,000	$0	$200,000
Study 6: Relating steelhead characteristics and environmental variables with marine survival trends	$31,403	$0	$0	$31,403
Study 7: Genome-wide association studies (GWAS) of survival and prevalence of pathogens and pathologies in steelhead smolts outmigrating through Puget Sound	$27,000	$0	$22,002	$49,002
Study 8: Hatchery Coho Telemetry Study	$29,000	$0	$0	$29,000
Study 9: Lipid content analysis and next steps asessing contaminants in steelhead	$0	?	$0	$0
Project coordination, communications, outreach	$69,000	$70,000		$139,000
Indirect	$57,351			$57,351
Total	$876,000	$632,154	$211,020	$1,719,174

Revenue	
Washington State Appropriation	$800,000
Nisqually Tribe	$20,000
Long Live the Kings	$27,000
Squaxin Tribe (for affiliated coho study that also satisfy needs of study 8)	$29,000

APPENDIX A: STUDY DESCRIPTIONS,

Study 1: Quantifying juvenile steelhead in harbor seal diet using scat analysis

Investigators: Austen Thomas (Smith Root) and Steve Jeffries (Washington Department of Fish and Wildlife)

Summary

It is currently hypothesized that harbor seal predation is a mechanism responsible for low juvenile steelhead survival in Puget Sound during steelhead outmigration. Acoustic telemetry work conducted in Puget Sound and Hood Canal has produced indirect evidence suggesting that harbor seals are consuming outmigrating juvenile steelhead in the marine environment, but we currently lack direct evidence of steelhead predation by harbor seals during the critical smolt outmigration time period. This study aims to provide direct evidence by identifying and quantifying steelhead DNA in fecal samples of Puget Sound harbor seals collected during the outmigration window. 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. These data, combined with quantification of all other prey species, will yield a percentage of seal population global diet comprised of juvenile steelhead. Those percentages can then be merged with seal bioenergetic data and a population census to estimate the biomass of juvenile steelhead (or number of individuals) consumed by harbor seals in Puget Sound. The advantage of this approach is that it eliminates doubt regarding the proximate source of mortality for juvenile steelhead, and serves as a complimentary validation for telemetry-based seal predation estimates.

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 steelhead in 3 sub-regions of Puget Sound, producing data useful for estimating the numbers of steelhead consumed by seals.

Objective 3 – Compare scat-based estimates of harbor seal predation to telemetry-based estimates of predation as a means of validation for both methods.

Study Design

Scat sampling

Puget Sound will be divided into three separate sub-regions for the purposes of scat collection and seal consumption estimates (Figure 1). These geographical sub-regions are based the distribution of acoustic tag receiver arrays used to estimate steelhead survival, and in addition, they are associated with waterway constrictions. Steelhead survival (and mortality) in each sub-region is currently estimated based on the percentage of acoustically tagged fish that successfully pass the receiver arrays. Therefore, by using a common geographical stratification scheme we can directly compare steelhead survival/mortality rates to scat-based harbor seal predation rates in each stratified sub-region, and compare scat-based seal mortality estimates to telemetry-based predation rates.

Scats will be collected every 10 days to 2 weeks between mid-March and mid-June, for a total of 8 collections, targeting low-tide temporal windows when appreciable numbers of scats can be acquired. We will strive to collect 70 harbor seal scat samples from seal haulout sites in each sub-region during the 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).

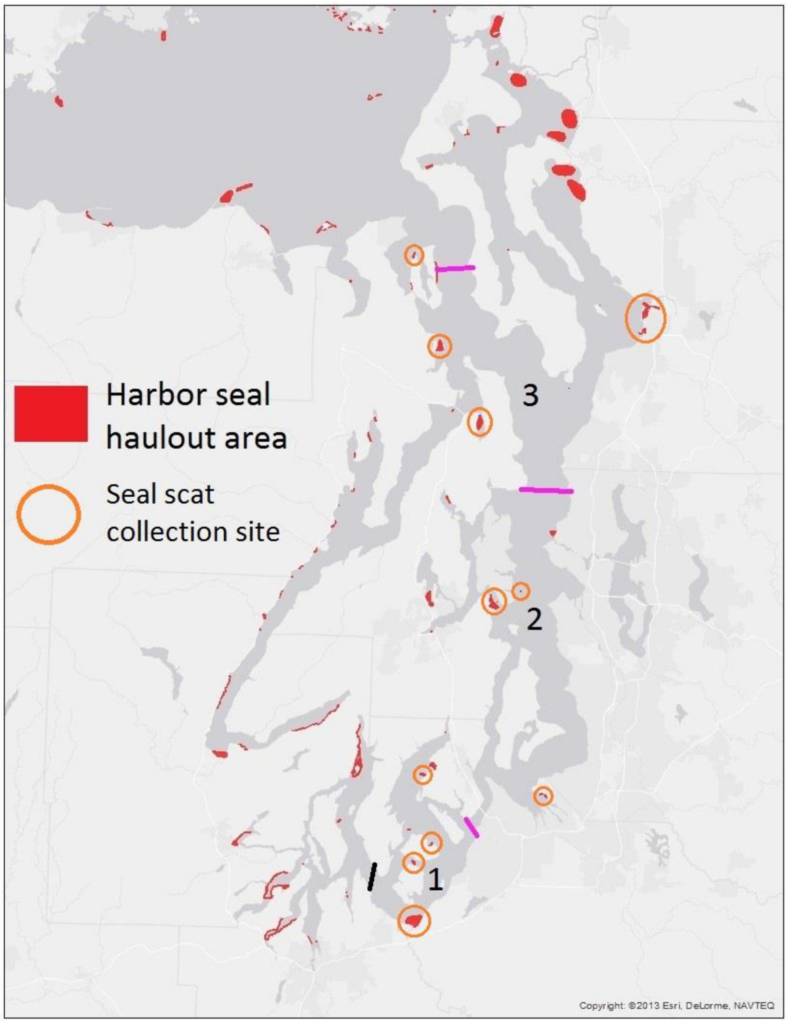


Figure 1. Study area depicting the geographically stratified sub-regions (1, 2, 3), and the seal haulout areas in each sub-region where scat samples can be collected. Pink lines indicate the acoustic tag receiver arrays used to estimate smolt survival that are also used as the sub-region boundaries for the scat study. Black line simply indicates the southern study area boundary.

At the haulout sites, each individual scat sample will be collected using a disposable wooden tongue depressor and placed in a 500ml Histoplex jar lined with a 126µm nylon mesh paint strainer (Orr *et al.* 2003). Samples will either be preserved immediately in the field by adding 300ml 95% ethanol to the collection jar, or 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 hard parts 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).

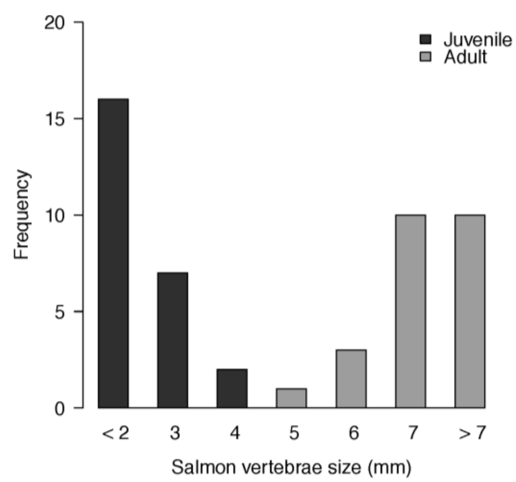


Figure 2. (From Thomas 2015) Frequency of salmon vertebrae between <2 mm and >7 mm, demonstrating the size difference between adult and juvenile salmon bones in seal scats.

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).

Collaborators at WDFW and NMFS will use the resulting percentage of juvenile steelhead in harbor seal diet (combined with seal population size and energy requirements) to estimate the numbers of juvenile steelhead eaten by seals in each sub-region. Lastly, comparisons will be made between the seal-related steelhead mortality rate (based on scatological analysis) and the survival of steelhead populations in each sub-region studied. Scat-based estimates of steelhead mortality from seals will also 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 in Puget Sound, and will provide an estimate of seal population diet comprised of juvenile steelhead. The data produced by this work will be used to compare estimates of seal predation based on indirect means (i.e. telemetry studies), and serve as an independent validation for those estimates.

Timeline

Activity	Start Date
Field work logistics	January 2016
Collection of seal scat samples	March – June 2016
Sample processing (hardparts)	July – October 2016
Sample processing (DNA metabarcoding)	July – October 2016
Data analysis (Bioinformatics + Diet %)	November – December 2016
Data analysis (Bioenergetics – NMFS)	December 2016 – January 2017
Reporting	February 2017

Deliverables

The deliverables from this study will be 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 steelhead in Puget Sound.

References

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Trites AW, Joy R (2005) Dietary analysis from fecal samples: how many scats are enough? *Journal of mammalogy* **86**, 704-712.

Study 2: Interactions between harbor seals and steelhead in Puget Sound

Investigators: Barry Berejikian (NOAA Fisheries), Megan Moore (NOAA Fisheries), and Steve Jeffries (Washington Department of Fish and Wildlife)

Overview

Studies conducted in 2014 provided the first information on temporal and spatial overlap between harbor seals and steelhead trout in Puget Sound, including inferred harbor seal consumption of steelhead smolts. Harbor seals outfitted with GPS tags and acoustic transceivers detected steelhead implanted with acoustic telemetry transmitters (tags) both during and after the smolt outmigration period. A total of 6,846 tag detections were recorded from 44 different steelhead trout smolts were recorded by 11 seal-mounted acoustic receivers. The 4 seal-mounted receivers monitored in Central Puget Sound (Orchard and Blakely Rocks) detected 37 of the 44 smolts (84%), and the 7 seal-mounted receivers in Admiralty Inlet detected 7 (16%) tagged smolts. Central Puget Sound seals detected a greater proportion of smolts surviving that far (29 of 50; 58%) than Admiralty Inlet seals (7 of 50; 14%; $P < 0.001$). Nine steelhead smolts were repeatedly detected by seal-mounted receivers when they were located at haulouts indicating the tags were defecated there by non-tagged harbor seals. Steelhead smolts tagged with silent tags (for 10 d) had very similar odds of survival through Puget Sound than smolts tagged with continuously pinging tags, providing no evidence that tag noise affected the survival of steelhead migrating through Puget Sound. Together with data from fixed arrays, we were able to categorize the fate of approximately one third of the steelhead entering Puget Sound as either having survived to Admiralty Inlet, stationary at a haulout location, or present in Puget Sound (and presumed stationary) after the smolt outmigration window.

Objectives

The primary objectives of this study are to estimate a predation rate by harbor seals on steelhead in Puget Sound (south of Admiralty Inlet), and determine whether predation by harbor seals differs by region. The first task will be to expand tag detection capabilities by i) monitoring harbor seals in South and Central Puget Sound and Admiralty Inlet, ii) place stationary receivers at seal haulouts and a random locations not frequented by harbor seals, iii) conduct mobile tracking to locate tags remaining in Puget Sound after the smolt outmigration period. The second task will be to quantify seal time at depth and locations in such a manner that estimates the amount of time seals spend at haulout locations. This information will be critical in estimating the probability that a tag consumed by a harbor seal would be defecated near a haulout site. The final task will be to incorporate data on harbor seal abundance, behavior, steelhead tag locations, and smolt abundance to estimate the predation rate and total number of smolts consumed by harbor seals.

Study design

Steelhead smolts will be captured at the WDFW rotary screw trap location in the Nisqually River (Fig. 1). Captured smolts will be held for one day before being anesthetized, weighed, measured (fork length) and implanted with a Vemco V7 2L acoustic transmitter (Vemco, Nova Scotia, Canada). Surgical implantation procedures are described in Moore et al. (in press). A total of 200 smolts will be tagged over a five 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 each river mouth. 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; Moore et al. in press)

Eighteen adult harbor seals will be captured (under Marine Mammal Protection Act Research Permit 13430) in April 2016 prior to the first smolt tagging. Each seal will be weighed, measured and fitted with an instrument pack that was glued to the pelage with quick-set Epoxy. Each pack will contain 1) a Vemco mobile transceiver (VMT) receiver capable of detecting both the V7 transmitters (69 kHz and transmissions from the VMTs, 2) a satellite-linked time depth recorder (TDR) and Fastloc GPS transmitter (model MK10AF, Wildlife Computers, Redmond, WA, USA, www.wildlifecomputers.com), 3) a VHF transmitter (164-165 MHZ, Advanced Telemetry Systems; www.atstrack.com) used for locating the instrument packs after they are shed by the harbor seals. All three instruments will be consolidated in a single floatation pack, which will be attached to the seal along the dorsal mid-line, on the anterior portion of the back. The GPS receivers will be programmed to transmit ARGOS and GPS data and to store Fastloc GPS locations on the tag every 10 min (three times more frequently than in 2014). Time and depth data will be recorded every 10 seconds. Only Fastloc GPS positions that incorporated data from 5 or more satellites will be used to minimize error (Hazel 2009). The VMTs will be continuously 'listening' for steelhead tags from the time of deployment until recovery.

In addition to monitoring the movements and tag detections by harbor seals, 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 (Berejikian et al. in prep). 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 towed through Puget Sound to identify the locations of stationary tags. 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. Preliminary feasibility studies are being conducted in summer 2015.

Data analysis

We will use the accurate timestamps provided by the GPS units and VMT receivers to 'associate' VMT detections of tagged steelhead with the detecting seal location, and thereby estimate the location of the steelhead tag. We will merge the Fastloc GPS timestamp data for a particular seal with the VMT timestamp data for steelhead tags detected by the same seal and calculated the minimum time differences (lag) between each VMT detection of a steelhead tag and the detecting seal's GPS location. To empirically determine VMT-GPS time associations that provide reasonably precise tag location information for the monitored seals in this study, we will deploy stationary 'sentinel' tags near the Gertrude Island, Orchard Rocks, Blakely Rocks and Colvos Rocks haulout locations. The distance between a GPS location of the seal within a specified time interval and the known sentinel tag location will provide an estimate of the error in the actual location of a VMT detection associated with a specific lag (i.e., time between VMT detection and GPS location). We may attempt to interpolate locations when VMT detections occur between two GPS locations as has been done in other studies (e.g., Lidgard et al. 2014) for detections in which the Fastloc GPS location frequency is sufficient.

To investigate spatial variation in detections of Nisqually River steelhead throughout the main basin of Puget Sound, we will use G-tests of independence (Sokal and Rohlf 2012) test whether the detection history (detected or not) of stationary tags near haulouts in South Puget Sound, Central Puget Sound and Admiralty Inlet is independent of estimated number of tagged smolts surviving to each region. To do this, we will first estimate the number of smolts released into the Nisqually River that survive to the vicinity of each of the tagging haulouts from segment-specific instantaneous mortality by distance estimates (number of mortalities/km) based on (Moore et al. in prep). We'll use shortest, straight line distances between the Nisqually River mouth and each haulout location to estimate mortalities/km and the number of smolts surviving to each region.

Predation events will be inferred from stationary tags detected near haulout locations by seal-mounted VMTs and stationary VR2 receivers. Other tag behavior data will be used to provide additional insight into whether or not an individual tags may have been consumed by harbor seals (see Berejikian et al. in review). The implicit assumption is that tags deposited near harbor seal haulouts were consumed by harbor seals and defecated there. Testing this assumption will involve monitoring non-haulout locations for the presence of stationary tags with fixed receivers and a mobile hydrophone. The number and proportion of Nisqually River smolts consumed by harbor seals in Puget Sound will be estimated from several variables 1) the number of harbor seals at monitored haulouts during the smolt migration window, 2) the total number of harbor seals in the study area (main basin of Puget Sound) 3) number of tags detected stationary at haulout locations (Berejikian et al. in prep), 4) proportion of time seals spend near (e.g., within 1 km) their haulout locations, and 5) estimated number of smolts entering Puget Sound from the Nisqually River.

Several analytical approaches exist for analyzing these types of tagging and telemetry data. We will initially construct a hierarchical and spatially explicit model to estimate the fates (and uncertainties in estimates) of the 200 tagged smolts (Royle and Dorazio 2008). This framework will allow us to estimate survival rates between arrays, detection probabilities, as well as predation risk. We will use a multinomial model to estimate the state of each fish in each time step, including whether the tags are stationary near haulout locations or not. This occupancy modeling framework is flexible, and allows covariates to be included in each component – for example the probability of becoming stationary (assumed to represent a fish that was consumed and the tag was defecated near the haul out site) might be modeled as a function of (1) the distance between the last known location and the nearest haulout and (2) the predation risk in the vicinity of the tag. Predation risk could be defined as simply being proportional to the number of seals present, however more complicated approaches could involve estimating predation risk maps from haulout estimates and movement tracks (Ward et al. 2012).

Outcomes

The study will provide temporal and spatial information on inferred predation by harbor seals on steelhead smolts and the information necessary to estimate predation rates on steelhead smolts from the Nisqually River entering south Puget Sound. The increase in spatial coverage, optimized programming of GPS tags, deployment of VR2W receivers, and mobile tracking will allow us to resolve the fate of a far greater percentage of tagged steelhead than in 2014. Documenting spatial patterns in predation will help to focus the location of potential management actions.

Time Line

Activity	Start Date
Permitting	July 2015
Purchase receivers, tags, and tagging supplies	December - January 2016
Deploy stationary receivers	March 2016
Capture seals and deploy instrument packs	March-April 2016
Collect, tag, and release smolts	April-May 2016
Tag and release steelhead smolts	April-June 2016
Retrieve/download receivers + mobile tracking	July 2016
Recover seal packs	August-September 2016
Data analysis	September 2016 -March 2017
Reporting	May 2017

Deliverables

Results of the telemetry study will be summarized and submitted for publication to a peer-reviewed scientific journal by July 2017. Data will be discussed at Salish Sea Marine Survival Workgroup meetings, presentations will be made at appropriate conferences and symposia, to WDFW, tribal co-managers, and interested stakeholders upon request.

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Royle JA and Dorazio RM (2008) Hierarchical modeling and inference in ecology. Academic Press.

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Study 3: Spring assessment of harbor seal numbers and distribution in Puget Sound

Principal Investigator: Steve Jeffries (Washington Department of Fish and Wildlife)

The principal investigator and scientific technician will be based in Tacoma with survey plane and pilot from Rite Bros. Aviation in Port Angeles.

Personnel will fly surveys in a small fixed-, high winged aircraft (Cessna 206) at an altitude of 600 to 800 feet. Surveys will be flown around daily low tides targeting a surveys window between two (2) hours before low tide to two (2) hours after low tide, when maximum numbers of harbor seal are known haul out. For each flight, WDFW will use the aircraft, pilot, and two observers (one mission commander for navigation, estimates and photography and one flight data recorder for data recording). Each survey with require about 3.5 hours of flight time to complete.

One flight would be flown each month April to June. All known haulout sites will be surveyed, and any new haulout sites will be identified during each flight. Seals or sea lions in the water will be recorded when encountered. Data collected during surveys will include date, time, and location, as well as a visual best estimate of seal numbers. Photographs will be collected of all sites where more than twenty-five (25) seals are hauled out. During the winter survey, the survey team will concentrate on collecting data for sea lion haulout sites due to the lack of low tides needed for exposure of intertidal sites used by harbor seals.

Aerial photographs will be taken of major haulout sites by WDFW. Digital photos will be taken with a handheld 35-millimeter (mm) Nikon digital camera with a 200 mm lens and shutter speeds of 1/500 to 1/1000 seconds. The digital images from the aerial photos will be counted to record total seal (and pups) and sea lion numbers for each site. Methodologies will be consistent with Jeffries et al. (2002). Counts will be entered into the WDFW seal and sea lion aerial survey database.

Study 4: Effects of Nanophyetus on swimming performance and survival of steelhead smolts

Investigators: Paul Hershberger (US Geological Survey), Martin Chen (Contractor), Barry Berejikian (NOAA Fisheries), Megan Moore (NOAA Fisheries)

Overview

Steelhead smolts originating in Puget Sound streams experience high mortality rates (approximately 80%) from their river mouths to the Pacific Ocean. The specific factors influencing mortality of migrating steelhead remain unknown, although it is clear from telemetry studies that mortality is rapid (within 1-2 weeks of seawater entry), occurs primarily in the marine environment (Moore et al., in review), and some is caused by pinniped predation (Berejikian et al. in prep). *Nanophyetus salmonica* infections may cause direct, disease-related mortality or indirect mortality by compromising the physical condition of migration steelhead smolts, thereby predisposing them to a greater risk of predation.

Recent fish health assays indicate high infection prevalence (87-100%) and intensity (800-2500 cysts/fish)of *Nanophyetus salmonica* in steelhead outmigrating from Central (Green) and South (Nisqually) Puget Sound Rivers. South and Central Puget Sound steelhead populations generally experience higher early marine mortality rates than those from North Puget Sound rivers, where Nanophyetus infections in assayed steelhead were absent (Chen et al. in preparation).). Steelhead are exposed to Nanophyetus as outmigrating juveniles after an infectious stage of the parasite is shed into the water column from the intermediate snail host.

The extent to which the *Nanophyetus* infections contribute to high observed mortality rates during the early marine phase of the juvenile steelhead life history remains unclear. High infection intensity among freshwater outmigrants could contribute to rapid mortality shortly after seawater entry (Jacobson et al. 2008) especially considering the decreased swimming performance of *Nanophyetus*-infected individuals (Butler and Millemann 1971). *Nanophyetus* is most virulent to salmonids during the early penetration and tissue migration phase (Baldwin et al. 1967) when the migrating cercarria cause significant disruption to host tissues. Salmonids with these early-stage *Nanophyetus* infections demonstrate reduced swimming speed (Butler and Milleman 1971). Afterwards, the parasite develops into a less-virulent metacercaria stage that encysts within the host tissues. A direct survival comparison of infected and healthy steelhead is needed to quantify and assess the effects of *Nanophyetus* infection on steelhead survival.

Objectives

We propose to use acoustic telemetry to evaluate differences in the early marine survival (near river mouth to Pacific Ocean) of specific pathogen-free (SPF) and *Nanophyetus*-infected Puget Sound steelhead smolts. Four acoustic receiver lines are currently functioning throughout the migratory path starting in South Puget Sound (Figure 1). Survival differences will be assessed through four separate migration segments within Puget Sound (Release-NAR, NAR-CPS, CPS-ADM, and ADM-JDF; Figure 1). This approach will help us understand effects of *Nanophyetus* infection at different stages of the steelhead smolt migration through Puget Sound. The study will also test whether *Nanophyeuts* infections affect swimming ability and survival after seawater transfer. The study will specifically test the following null hypotheses:

1) H_0: *Nanophyetus* infection does not affect smolt survival during natural migration through Puget Sound
2) H_0: *Nanophyetus* infection does not affect survival after transfer to seawater in a controlled (laboratory) environment
3) H_0: Nanophyetus infection does not affect swimming performance
4) H_0: Tagging with acoustic telemetry transmitters does not differentially affect the survival of infected and non-infected steelhead smolts after seawater transfer.

Study design

I. Relative Survival of Nanophyetus-infected and –uninfected steelhead.

The proposed study would utilize a total of 460-560 hatchery-raised steelhead smolts for a combination laboratory/field experimental design. The specific-pathogen-free (SPF) status of hatchery steelhead will be confirmed prior to experiment initiation by subsampling individuals from the source hatchery population. SPF experimental animals will be transferred to the U.S. Geological Survey – Marrowstone Marine Field Station. All *Nanophyetus* exposures will occur by immersion of steelhead in cercarria suspensions; negative controls (uninfected) will be handled in an identical manner, but not exposed to cercarria. *NOTE: Using an eDNA approach, a quantitative polymerase chain reaction (qPCR), targeted against specific regions of the Nanophyetus salmincola genome, is currently being developed. This qPCR will be used to determine the waterborne Nanophyetus cercarria exposure levels in this experiment.*

To assess the effects of *Nanophyetus* on the survival of steelhead released into Puget Sound, we will track the outmigration of infected and uninfected individuals using hydroacoustic tags. All smolts (N=300) will be implanted with V7 Vemco acoustic transmitters (69 kHz, 7 mm diameter, 18 mm length, 1.0 g (Table 1). Half of the tagged fish will then be exposed to *Nanophyetus*; controls will remain unexposed. Details of the challenge model will be developed in rainbow trout surrogate hosts prior to full implementation in steelhead. A group of 150 infected, tagged smolts will be released along with a group of 150 non-infected, tagged smolts directly into Puget Sound. Detections of acoustic telemetry tags on four currently deployed acoustic receiver lines (CPS, ADM, JDF; Figure 1) will be used to populate Cormack-Jolly-Seber mark-recapture models (Lebreton et al. 1992) which will ultimately be used to compare survival of the infected and non-infected release groups.

To assess the effects of *Nanophyetus* on the ability of steelhead to survive seawater transition, groups of infected and uninfected smolts (N=30 each) will be acclimated to seawater at the Marrowstone Marine Field Station. Further, to assess any effects of surgery on infection prevalence or intensity, 60 additional steelhead will receive dummy transmitters (identical in form to actively pinging transmitters); half will then be exposed to *Nanophyetus*. Survival (30 days post-seawater transition), infection prevalence, and parasite load will be compared among all 4 groups:

- Tagged (infected and uninfected)
- Untagged (infected and uninfected)

Table 1. Allocation of hatchery steelhead smolts to Nanophyetus exposures and assays.

Disposition	Exposed	Non-exposed
Tagged and released	150	150
Dummy-tagged for seawater challenge	30	30
Non-tagged for seawater challenge	30	30
Non-tagged for swim test	20	20

II. Swimming Performance of Nanophyetus-infected steelhead

The effects of acoustic tag insertion and *Nanophyetus* infections on the swimming performance of steelhead will be assessed in a Blazka-type respirometer. Briefly, the time-to-fatigue will be assessed in 20 experimental fish from each treatment group (infected and uninfected) in a ramped exercise regime where water velocity is progressively increased at predetermined time points. The effects of infection progression on swimming performance will be assessed by comparing the swimming performance of fish on days 3, 14, and 30 post-infection. Mean time-to-fatigue will be compared the two groups.

Outcomes

This combination laboratory and field study will provide a good comparison of the mortality patterns experienced by steelhead smolts with and without *Nanophyetus* infections. With the receiver detection data we will be able to estimate survival through four different migration segments. The temporal pattern of mortality will be compared between groups, and will help us understand when the pathogen may be the most detrimental. If there is no difference in survival between survival of the infected and uninfected groups we will be able to largely discount the effect of *Nanophyetus* on steelhead survival and focus on the study of other possible mortality mechanisms.

Time Line

Activity	Start Date
Identify hatchery stock	Summer 2015
Purchase tags, and tagging supplies	January 2016
Transfer smolts from hatchery	April 2016
Implant smolts with transmitters	April 2016
Nanophyetus challenge	May 2016
Release tagged smolts	May 2016
Swimming performance assay	May-June 2016
qPCR analysis	May 2016
Data analysis	September 2016
Reporting	January 2017

Deliverables

Results of the *Nanophyetus* studies will be summarized and submitted for publication to a peer-reviewed scientific journal by July 2017. Data will also be presented orally to interested parties and at relevant scientific meetings.

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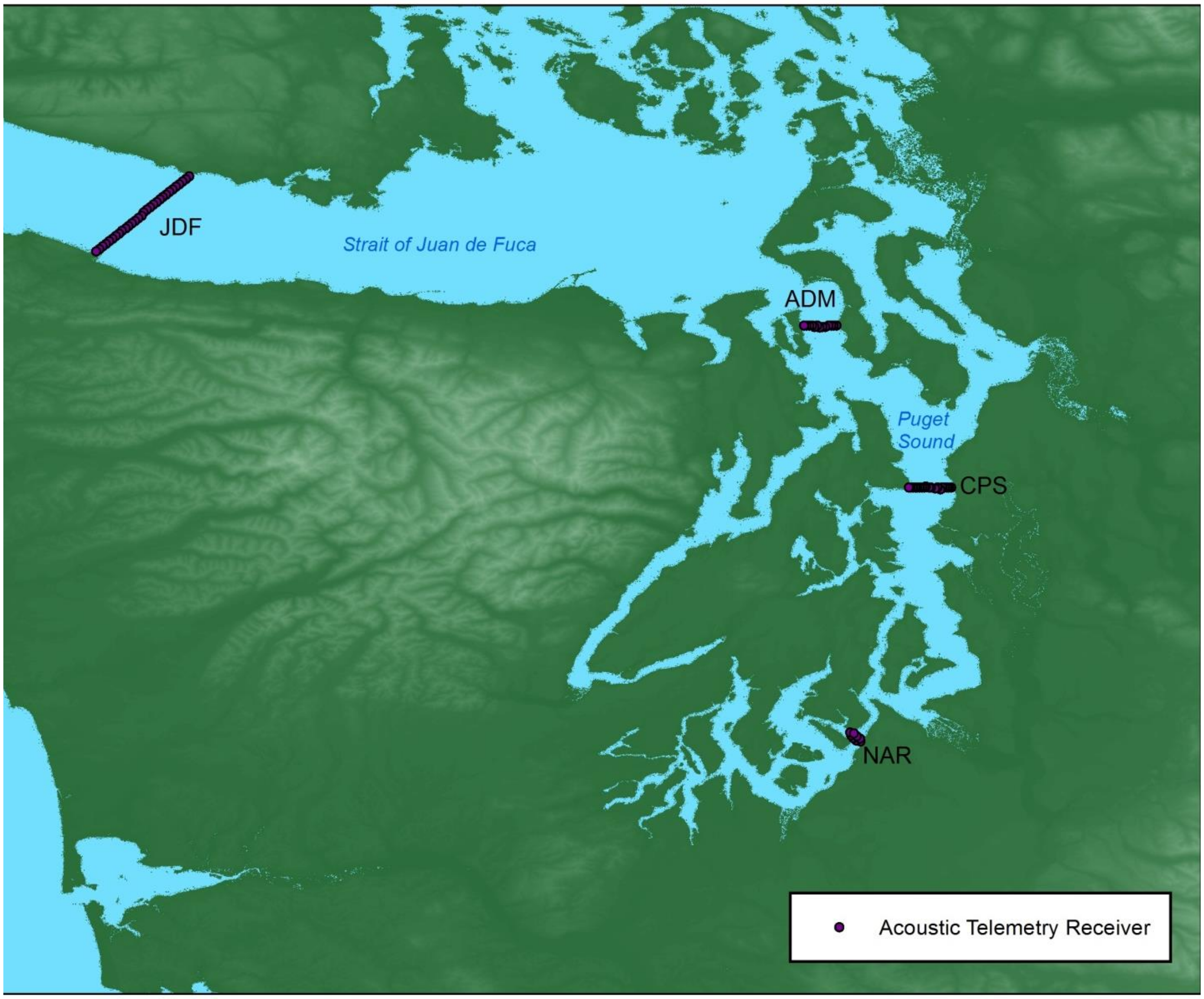

Figure 1. Map of study area showing currently deployed telemetry receiver arrays at the Tacoma Narrows (NAR), Central Puget Sound (CPS), Admiralty Inlet (ADM), and the Strait of Juan de Fuca (JDF).

Study 5: Toward management of *Nanophyetus* - Assessing *Nanophyetus* exposure zones - to be performed if additional USGS funds are available

Principal Investigator: Paul Hershberger (US Geological Survey)

This study involves some forward thinking and its results will be most pertinent if we find that *Nanophyetus* does contribute to higher early marine mortality rates via Study 4, above.

The current recovery steelhead efforts in Puget Sound are largely focused towards developing tangible restoration options, efforts intended to mitigate any deleterious impacts of *Nanophyetus* should be structured around an adaptive disease management approach. The most likely approach for mitigating impacts of *Nanophyetus* to steelhead will involve some type of active intervention to the free-swimming cercarria (i.e infectious) life stage of the parasite. Restoration options may include:

- Timing the release of hatchery fish to be non-coincident with cercarria shedding events from infected snails.
- Transporting outmigrating fish around known *Nanophyetus* exposure zones.
- Treating endemic watershed to remove the infected snails or the free-swimming cercarria.

Prior to recommending or implementing any of these adaptive disease management strategies, a better ecological understanding of *Nanophyetus* is needed, particularly regarding the factors that influence infection pressures in endemic watersheds.

Using an eDNA approach, a quantitative polymerase chain reaction (qPCR), targeted against specific regions of the *Nanophyetus salmincola* genome, is currently being developed. Once developed and optimized, this tool will be used to:

- Assess the diurnal, seasonal, and longitudinal details of Nanophyetus shedding in endemic watersheds.
- Assess the geographical and watershed-wide distributions of Nanophyetus-positive Juga spp. snails throughout the region.

By detecting *N. salmioncola* in water and snail samples at different times and locations throughout the watersheds, we will identify:

- Temporal windows of opportunity when out-migrating steelhead might avoid *Nanophyetus* exposures.
- Spatial infectious zones in affected watersheds that should be avoided, possibly with steelhead transplantation efforts.

Study 6: Relate steelhead characteristics and environmental variables with smolt marine survival trends

Principal Investigator: Neala Kendall (Washington Department of Fish and Wildlife)

Justification

The portfolio effect suggests that diversification minimizes the volatility of an investment and describes why more diverse systems are more stable (Lehman and Tilman 2000, Koellner and Schmitz 2006, Schindler et al. 2010). In this way, diverse life history characteristics of steelhead may buffer their abundance and productivity rates. By evaluating which individual fish and population traits are most correlated with smolt marine 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). Additionally, environmental conditions steelhead face in both their freshwater and marine environments also likely influence, either directly or indirectly, smolt survival in the ocean. If freshwater processes are more closely related to smolt survival trends than marine processes, this suggests that lower smolt survival trends may be driven by fish being weaker as they enter the marine environment. On the other hand, stronger correlations of smolt survival with conditions and processes in the marine environment shift the evidence towards that stage limiting steelhead abundance and productivity.

Background and hypotheses

We will analyze existing data on wild and hatchery summer- and winter-run steelhead individual and population characteristics and environmental data and relate these values to steelhead smolt-to-adult survival (SAR) trends 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. Ideally, this effort would also include developing new data collection stations for additional populations or regions into the future. The goal of this work would be to evaluate the relationship between SAR differences and 1) variation in population life-history diversity and 2) the physical environment to which steelhead are exposed are associated. We would examine hypotheses related to spatial variation in mortality, size-selective mortality, match-mismatch, and life history variation. We will address the following questions:

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

We hypothesize that SAR data are correlated with changes in hatchery and wild steelhead and salmon smolt abundance. A positive correlation (e.g., higher SARs 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 SARs relative to lower smolt abundance may indicate predator disinterest.

2. Are SAR trends correlated with population life-history diversity?

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

3. Which ecosystem indicators best predict steelhead early 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.

4. Are SAR trends correlated with changes in buffer prey abundance?

We hypothesize that, in years when and where more herring (and other buffer prey species) were present (and when they were present at larger sizes), steelhead survived at a higher rate in the Puget Sound marine environment.

Materials and methods

SAR data for Puget Sound, coastal Washington and Oregon, and lower Columbia River (and potentially the Keogh River of BC, Canada, but the inclusion of these data remains uncertain) will be statistically analyzed and correlated with the variables listed above. Such analysis will help determine whether certain characteristics or conditions are contributing uniquely to mortality (or are uniquely affected by the environment) in Puget Sound. For example, we will 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.

The following fish characteristics will be included in this analysis, when data are available: summer vs. winter run; 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; hatchery and wild smolt size; wild smolt outmigration timing; hatchery smolt release timing; smolt counts across salmonid species; and origin and migration patterns (from natal stream through Puget Sound). 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, predators, 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.

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 SARs), time series methods (to test for the presence of variations and patterns over time), and correlations (to examine relationships between 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, subregion, 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 SARs.

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 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 begin in 2016 and will continue through the biennium. This work will be written for peer-reviewed publication by June 2017.

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Study 7: Genome-wide association studies (GWAS) of survival and prevalence of pathogens and pathologies in steelhead smolts outmigrating through Puget Sound

Kenneth I. Warheit (Washington Department of Fish and Wildlife)

Overview

The purpose of this document is to outline briefly two proposed research projects that are logically connected and effective continuations of two projects previously funded through the Salish Sea Marine Survival Project – Puget Sound steelhead. First, I propose to add to the original Genome-wide Association Study (GWAS) the 2014 reciprocal transplant samples not included in the original GWAS, and the 2015 acoustically tagged samples from the Nisqually River. These additional samples will, presumably, increase statistical power to enable an appropriate test for the association between genomes and survival within a broadly defined population of outmigrating steelhead smolts. In addition, unlike the original GWAS where I defined fate absolutely as either successfully migrating through Puget Sound (survival) versus not being detected by any acoustic receiver (mortality), in this new project I will define fate incrementally, enabling the use of all samples with genomic sequences. Second, I proposal to add a genomic perspective to the Chen et al. (2015) study on the prevalence and load of *Nanophyetus salmincola* in outmigrating steelhead smolts. As in the first proposed study, I will conduct a GWAS on a set of samples from Chen et al. (2015); however, in this second study instead of testing for an association with survival, I will test for genomic association with the prevalence of primarily *Nanophyetus*, and secondarily myxosporean species, *Sanguinicola* spp, and gill and heart pathologies.

Objectives

This project has two components. First, in the original GWAS (Warheit et al. 2015) we detected four loci with significant association with survival. Three of these loci matched sequences in the National Center for Biotechnology Information (NCBI) database; one sequence matched morphogenesis genes, and another sequence matched a series of different genes with the majority of these genes having immunological function. However, the original GWAS lacked sufficient power to provide a definitive association between smolt genotype and fate: (1) sample sizes were too small and post-hoc test were not possible; (2) there was a lack of independence between year and source, and between source and release; and (3) I defined fate as a categorical phenotype while the model was built for quantitative data. In the first component of this new study, I will increase sample sizes, redefine survival, and redesign the study to address the limitations of the original GWAS. As with the original GWAS my objectives here are to test for genomic associations with steelhead smolt fate in Puget Sound, with:

H_0: no genomic association with steelhead smolt fate

H_1: significant association with at least one functional gene and steelhead smolt fate

In the second component of this project I will conduct a GWAS on a set of genomic samples collected by Chen et al. (2015) as part of their study. Here, instead of looking for a genomic association with steelhead survival, I will test for genomic association with the prevalence of *Nanophyetus salmincola*.

Although the main target for this analysis is *Nanophyetus*, I will also test for statistical associations with myxosporean species, *Sanguinicola* spp, and gill and heart pathologies, data already collected by Chen et al. (2015). For this component, my hypotheses are:

H_0: no genomic association with any pathogen or pathology

H_1: significant association with at least one pathogen (primarily *Nanophyetus*) or pathology

Study design

All samples for this project have been collected previously, so there is no field aspect to this research.

Steelhead survival GWAS – increasing power. I will increase sample sizes by genomic sequencing the 126 steelhead smolts tagged in 2014 but not included in the original GWAS, and 97 steelhead smolts tagged in 2015, for a total of 223 samples. I will limit the GWAS analysis to Green and Nisqually river samples from 2014 and 2015, removing the issue about lack of independence between year and source, and between source and release. In the original GWAS I defined fate as a binary character – survival if the individual was detected at the JDF acoustic array, and mortality if the individual was detected leaving freshwater, but not detected at any of the three focal acoustic arrays (Narrows [for Nisqually] or CPS [for Green], Admiralty, and JDF). All other individuals were excluded from the analysis. For this new project I define fate as a quasi-continuous phenotype: 1.00 if detected at JDF array, 0.67 if detected at Admiralty but not at JDF, 0.33 if detected at Narrows or CPS, but not at Admiralty and JDF, and 0.00 if detected leaving freshwater but not detected at Narrows or CPS, Admiralty, and JDF. Redefining fate allows the use of all individuals leaving freshwater, thereby greatly increasing sample sizes. There are a total of 203 samples from the 2014 reciprocal transplant study. Eighteen of these samples were not detected leaving freshwater, giving a total of 185 samples from 2014 to be included in the analysis (126 to be sequenced as part of this new project) (Table 1). There are 97 individuals from 2015 that will be sequenced and included in the analysis, giving a total of 282 samples from Green and Nisqually rivers in 2014 and 2015 to be analyzed, compared with only 59 in the original GWAS.

Steelhead pathogens and pathologies GWAS. The laboratory work for this component of the study is identical to that of the previous component. I selected 245 samples from Green and Nisqually rivers with *Nanophyetus salmincola* cysts counts and gill, liver, heart, and kidney histology data (data from M. Chen; Table 2). These samples will be processed and sequenced along with the 223 samples described above, for a total of 468 samples.

Genomic sequencing, bioinformatics, and statistical analyses. The 468 samples from both components will be processed simultaneously as five genomic libraries. As described in Warheit et al. (2015), each sample will be sequenced using restriction-site associated DNA (RAD) sequences or RAD-tags (RAD-seq) (Miller et al. 2007, Baird et al. 2008, Davey et al. 2011). RAD-seq libraries will be prepared at the WDFW's Molecular Genetics Laboratory, and sent to the University of Oregon Genomics Core Facility for sequencing using an Illumina HiSeq2000 sequencer. RAD sequences from all 468 samples will be processed together using the program STACKS (Catchen et al. 2011, Catchen et al. 2013) to identify homologous RAD-tags, to generate an initial list of SNPs, and to genotype all individuals at these SNP loci. Following this bioinformatics pipeline the combined dataset will be divided into their respective components and analyzed separately. As with the original GWAS, I will use the mixed linear model (MLM) procedure in the program TASSEL (Yu et al. 2006, Bradbury et al. 2007, Zhang et al. 2010) to test for associations between genotypes and fate (component 1), and pathogens and pathologies (component 2).

Outcomes

In the first component of this project I am testing for a genomic association with fate (survival). In the second component, I am testing for a genomic association with pathogens and pathologies. Although I will be examining the same set of genes in both components, the two components make use of different sets of individuals. However, my logic in linking these two components is as follows: If these pathogens or pathologies directly or indirectly (though predation, for example) affect survival I should find in both components a similar set of genes with significant associations. If this occurs it suggests that at least for the Nisqually and Green rivers, steelhead smolt survival is linked statistically to pathogens or pathologies, and not random with respect to an individual's genome. This would also suggest that these pathogens and potentially an individuals' immunological response to these pathogens, for example, play a role in steelhead smolt survival, and possibly the viability of these specific steelhead populations.

Time Line

Since the two components of this project differ only in what metadata are included in the statistical analyses, I have considered together both components into a single time line

Activity	Start Date
Organize samples and metadata	December 2015
Extract and quantitate DNA, and select final sample run list	January 2016
RAD library preparation – 5 libraries	February 2016
RAD sequencing – contract at University of Oregon	March 2016
Bioinformatics	April – May 2016
Data analysis	June – September 2016
Reporting	November 2016

Deliverables

Progress reports and final analyses will be discussed at Salish Sea Marine Survival Project steelhead workgroup and technical team meetings. In addition, the results will be presented at scientific meeting, and where applicable, co-manager meetings. A final written report on the results and conclusions of the project will be completed and submitted by November 2016. If appropriate, the report will be re-designed as a manuscript and submitted for publication in a peer-reviewed journal no later than February 2017.

Budget

Three options were presented for consideration: (1) a single project with two components; (2) expansion of the original GWAS, including the 2014 and 2015 tagged individuals from Green and Nisqually rivers (component 1); and (3) steelhead pathogens and pathologies GWAS. The cost for bioinformatics, statistical analyses, and report and manuscript preparation is $10,000 for any of the three options. The total cost, including indirect for a single component (either 1 or 2 – options 2 or 3) is $34,881.53; however, the total cost for both components as an integrated single project (option 1) is $49,001.60. In other words, considering both projects together adds only $14,120.07 to the cost of a single project.

Budget

Category	Option 1 Components 1 & 2 5 Libraries 468 samples	Option 2 Component 1 3 Libraries 223 samples	Option 3 Component 2 3 Libraries 245 samples
RAD library preparation and sequencing			
Consumables and UO contract	$24,324	$14,596	$14,596
Salary and Benefits	$3,600	$2,400	$2,400
Bioinformatics, statistics, reporting			
Salary and Benefits	$10,000	$10,000	$10,000
Subtotal	$37,924.00	$26,996.00	$26,996.00
Indirect (29.21%)	$11,077.60	$7,885.53	$7,885.53
Total	**$49,001.60**	**$34,881.53**	**$34,881.53**

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Table 1. Sample list for Component 1 – testing genomic associations with survival

RAD Genotyped Original GWAS	Final Analysis Original GWAS	To Be RAD Genotyped New Project	Original GWAS MGL Code	Source Population	Release Population	Detection at				Original Survival Score	New Mortality Ranking
						RM	Narrows or CPS	Admiralty	JDF		
Yes	Yes	No	14GM0008	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0045	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0050	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0053	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0085	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0092	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0097	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0044	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0094	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0047	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0048	Green	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0003	Green	Green	1	1	1	1	Survival	1
Yes	Yes	No	14GM0007	Green	Green	1	1	1	1	Survival	1
Yes	Yes	No	14GM0051	Green	Green	1	1	1	1	Survival	1
Yes	Yes	No	14GM0056	Green	Green	1	1	0	1	Survival	1
Yes	Yes	No	14GM0090	Green	Green	1	1	1	1	Survival	1
Yes	Yes	No	14GM0091	Green	Green	1	1	0	1	Survival	1
Yes	Yes	No	14GM0093	Green	Green	1	1	0	1	Survival	1
Yes	Yes	No	14GM0027	Green	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GM0079	Green	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GM0010	Green	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GM0058	Green	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GM0019	Green	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GM0011	Green	Nisqually	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0072	Green	Nisqually	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0037	Green	Nisqually	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0086	Green	Nisqually	1	0	0	0	Mortality	0
Yes	Yes	No	14GM0099	Green	Nisqually	1	1	1	1	Survival	1
Yes	Yes	No	14GM0036	Green	Nisqually	1	1	1	1	Survival	1
Yes	Yes	No	14GN0110	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0073	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0003	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0006	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0040	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0047	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0079	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0115	Nisqually	Green	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0082	Nisqually	Green	1	1	1	1	Survival	1
Yes	Yes	No	14GN0010	Nisqually	Green	1	0	1	1	Survival	1
Yes	Yes	No	14GN0038	Nisqually	Green	1	0	0	1	Survival	1
Yes	Yes	No	14GN0112	Nisqually	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GN0116	Nisqually	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GN0007	Nisqually	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GN0009	Nisqually	Green	0	0	0	0	Mortality	RiverMort
Yes	Yes	No	14GN0018	Nisqually	Nisqually	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0122	Nisqually	Nisqually	1	0	0	0	Mortality	0
Yes	Yes	No	14GN0060	Nisqually	Nisqually	1	1	1	1	Survival	1
Yes	Yes	No	14GN0025	Nisqually	Nisqually	1	1	1	1	Survival	1
Yes	No	No	14GM0031	Green	Green	1	0	0	0	Mortality	0
Yes	No	No	14GM0078	Green	Green	1	0	0	0	Mortality	0
Yes	No	No	14GM0009	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0014	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0029	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0049	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0055	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0059	Green	Green	1	1	1	0	NA	0.67

RAD Genotyped Original GWAS	Final Analysis Original GWAS	To Be RAD Genotyped New Project	Original GWAS MGL Code	Source Population	Release Population	Detection at				Original Survival Score	New Mortality Ranking
						RM	Narrows or CPS	Admiralty	JDF		
Yes	No	No	14GM0060	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0080	Green	Green	1	1	1	0	NA	0.67
Yes	No	No	14GM0006	Green	Nisqually	1	1	1	0	NA	0.67
Yes	No	No	14GM0082	Green	Nisqually	1	1	1	0	NA	0.67
Yes	No	No	14GN0072	Nisqually	Green	1	0	0	0	Mortality	0
Yes	No	No	14GN0002	Nisqually	Green	1	1	1	0	NA	0.67
Yes	No	No	14GN0011	Nisqually	Green	1	1	1	0	NA	0.67
Yes	No	No	14GN0037	Nisqually	Green	1	1	1	0	NA	0.67
Yes	No	No	14GN0039	Nisqually	Green	1	1	1	0	NA	0.67
Yes	No	No	14GN0043	Nisqually	Green	1	1	1	0	NA	0.67
Yes	No	No	14GN0074	Nisqually	Green	1	1	1	0	NA	0.67
Yes	No	No	14GN0078	Nisqually	Green	1	1	1	0	NA	0.67
No	No	Yes	NA	Green	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Green	1	0	1	0	NA	0.67
No	No	Yes	NA	Green	Green	1	0	1	0	NA	0.67
No	No	Yes	NA	Green	Green	1	1	1	0	NA	0.67
No	No	Yes	NA	Green	Green	1	1	1	0	NA	0.67
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33

No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Green	Nisqually	0	0	0	0	Mortality	RiverMort

RAD Genotyped Original GWAS	Final Analysis Original GWAS	To Be RAD Genotyped New Project	Original GWAS MGL Code	Source Population	Release Population	Detection at RM	Narrows or CPS	Admiralty	JDF	Original Survival Score	New Mortality Ranking
No	No	Yes	NA	Green	Nisqually	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Green	Nisqually	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Green	1	0	1	0	NA	0.67
No	No	Yes	NA	Nisqually	Green	1	0	1	0	NA	0.67
No	No	Yes	NA	Nisqually	Green	1	0	1	0	NA	0.67
No	No	Yes	NA	Nisqually	Green	1	1	1	1	Survival	1
No	No	Yes	NA	Nisqually	Green	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Nisqually	Green	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Nisqually	Green	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0

No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	0	0	0	Mortality	0
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33

RAD Genotyped Original GWAS	Final Analysis Original GWAS	To Be RAD Genotyped New Project	Original GWAS MGL Code	Source Population	Release Population	Detection at				Original Survival Score	New Mortality Ranking
						RM	Narrows or CPS	Admiralty	JDF		
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	1	1	0	0	NA	0.33
No	No	Yes	NA	Nisqually	Nisqually	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Nisqually	Nisqually	0	0	0	0	Mortality	RiverMort
No	No	Yes	NA	Nisqually	Nisqually	0	0	0	0	Mortality	RiverMort

Table 2. Sample list for Component 2 – testing genomic associations with pathogens and pathologies. Data courtesy of from M. Chen.

River	Location	Origin	Fish ID	Histology				Total Nano cysts
				Gill	Liver	Heart	Kidney	
Green	Hatchery	H	145417	Y	Y	Y	Y	4046
Green	Hatchery	H	145403	Y	Y	Y	Y	1574
Green	Hatchery	H	145401	Y	Y	Y	Y	610
Green	Hatchery	H	145404	Y	Y	Y	Y	1717
Green	Hatchery	H	145405	Y	Y	Y	Y	1823
Green	Hatchery	H	145406	Y	Y	Y	?	2043
Green	Hatchery	H	145407	Y	?	?	?	2121
Green	Hatchery	H	145427	Y	Y	Y	Y	5908
Green	Hatchery	H	145408	Y	Y	Y	Y	2155
Green	Hatchery	H	145412	Y	Y	Y	Y	3593
Green	Hatchery	H	145421	Y	Y	Y	Y	4342
Green	Hatchery	H	145419	Y	Y	Y	Y	4227
Green	Hatchery	H	145415	Y	Y	Y	Y	3846
Green	Hatchery	H	145429	Y	Y	Y	Y	8081
Green	Hatchery	H	145414	Y	Y	Y	Y	3712
Green	Hatchery	H	145423	Y	Y	Y	Y	4624
Green	Hatchery	H	145425	Y	Y	Y	Y	4932
Green	Hatchery	H	145413	Y	Y	Y	Y	3700
Green	Hatchery	H	145416	Y	Y	Y	Y	3871
Green	Hatchery	H	145428	Y	Y	Y	Y	6106
Green	Hatchery	H	145430	Y	Y	Y	Y	8357
Green	Hatchery	H	145420	Y	Y	Y	Y	4300
Green	Hatchery	H	145424	Y	Y	Y	Y	4796
Green	Hatchery	H	145411	Y	Y	Y	Y	3590
Green	Hatchery	H	145402	Y	Y	Y	Y	1268
Green	Hatchery	H	145409	Y	Y	Y	Y	2369
Green	Hatchery	H	145410	Y	Y	Y	Y	3371
Green	Hatchery	H	145418	Y	Y	Y	Y	4093
Green	Hatchery	H	145422	Y	Y	Y	Y	4569
Green	Hatchery	H	145426	Y	Y	Y	Y	5158
Green	Trap	H	145459	Y	Y	Y	Y	0
Green	Trap	H	145460	Y	Y	Y	Y	0
Green	Trap	H	145431	Y	Y	Y	Y	80
Green	Trap	H	145432	Y	Y	Y	Y	108
Green	Trap	H	145457	Y	Y	Y	Y	9
Green	Trap	H	145444	Y	Y	Y	Y	55
Green	Trap	H	145442	Y	Y	Y	Y	161
Green	Trap	H	145452	Y	Y	Y	Y	117
Green	Trap	H	145453	Y	Y	Y	Y	85
Green	Trap	H	145454	Y	Y	Y	Y	65
Green	Trap	H	145455	Y	Y	Y	Y	34
Green	Trap	H	145456	Y	Y	Y	Y	155
Green	Trap	H	145433	Y	Y	Y	Y	23
Green	Trap	H	145434	Y	Y	Y	Y	52
Green	Trap	H	145435	Y	Y	Y	Y	110
Green	Trap	H	145436	Y	Y	Y	Y	25
Green	Trap	H	145437	Y	Y	Y	Y	103
Green	Trap	H	145438	Y	Y	Y	Y	76
Green	Trap	H	145439	Y	Y	Y	Y	152
Green	Trap	H	145440	Y	Y	Y	Y	92

Green	Trap	H	145441	Y	Y	Y	Y	37
Green	Trap	H	145443	Y	Y	Y	Y	129
Green	Trap	H	145445	Y	Y	Y	Y	36
Green	Trap	H	145446	Y	Y	Y	Y	136
Green	Trap	H	145447	Y	Y	Y	Y	45

River	Location	Origin	Fish ID	Histology				Total Nano cysts
				Gill	Liver	Heart	Kidney	
Green	Trap	H	145448	Y	Y	Y	Y	73
Green	Trap	H	145449	Y	Y	Y	Y	65
Green	Trap	H	145450	Y	Y	Y	Y	119
Green	Trap	H	145451	Y	Y	Y	Y	54
Green	Trap	H	145458	Y	Y	Y	Y	0
Green	Trap	W	145468	Y	Y	Y	Y	655
Green	Trap	W	145474	Y	Y	Y	Y	0
Green	Trap	W	145461	Y	Y	Y	Y	0
Green	Trap	W	145462	Y	Y	Y	Y	0
Green	Trap	W	145463	Y	Y	Y	Y	23
Green	Trap	W	145464	Y	Y	Y	Y	0
Green	Trap	W	145465	Y	Y	Y	Y	0
Green	Trap	W	145466	Y	Y	Y	Y	0
Green	Trap	W	145467	Y	Y	Y	Y	887
Green	Trap	W	145470	Y	Y	Y	Y	0
Green	Trap	W	145471	Y	Y	Y	Y	0
Green	Trap	W	145472	Y	Y	Y	Y	0
Green	Trap	W	145473	Y	Y	Y	Y	0
Green	Trap	W	145475	Y	Y	Y	Y	0
Green	Trap	W	145478	Y	Y	Y	Y	0
Green	Trap	W	145469	Y	Y	Y	Y	0
Green	Trap	W	145477	Y	Y	Y	Y	1227
Green	Trap	W	145476	Y	Y	Y	Y	0
Green	Trap	W	145479	Y	Y	Y	Y	0
Green	Trap	W	145480	Y	Y	Y	Y	0
Green	Trap	W	145481	Y	Y	Y	Y	0
Green	Trap	W	145482	Y	Y	Y	Y	0
Green	Trap	W	145483	Y	Y	Y	Y	0
Green	Trap	W	145484	Y	Y	Y	Y	0
Green	Trap	W	145485	Y	Y	Y	Y	0
Green	Trap	W	145486	Y	Y	Y	Y	0
Green	Trap	W	145487	Y	Y	Y	Y	0
Green	Trap	W	145488	Y	Y	Y	Y	0
Green	Trap	W	145489	Y	Y	Y	Y	0
Green	Trap	W	145490	Y	Y	Y	Y	0
Green	Estuary	H	145498	Y	Y	Y	Y	100
Green	Estuary	H	145504	Y	Y	Y	Y	143
Green	Estuary	H	145515	Y	Y	Y	Y	1
Green	Estuary	H	145516	Y	Y	Y	Y	6
Green	Estuary	H	145528	Y	Y	Y	Y	363
Green	Estuary	H	145497	Y	Y	Y	Y	73
Green	Estuary	H	145505	Y	Y	Y	Y	1641
Green	Estuary	H	145506	Y	Y	Y	Y	1089
Green	Estuary	H	145507	Y	Y	Y	Y	542
Green	Estuary	H	145508	Y	Y	Y	Y	550
Green	Estuary	H	145509	Y	Y	Y	Y	560
Green	Estuary	H	145510	Y	Y	Y	Y	275
Green	Estuary	H	145511	Y	Y	Y	Y	208

Green	Estuary	H	145514	Y	Y	Y	Y	645
Green	Estuary	H	145491	Y	Y	Y	Y	177
Green	Estuary	H	145492	Y	Y	Y	Y	84
Green	Estuary	H	145493	Y	Y	Y	Y	145
Green	Estuary	H	145494	Y	Y	Y	Y	72
Green	Estuary	H	145495	Y	Y	Y	Y	138
Green	Estuary	H	145496	Y	Y	Y	Y	75
Green	Estuary	H	145499	Y	Y	Y	Y	164

River	Location	Origin	Fish ID	Histology				Total Nano cysts
				Gill	Liver	Heart	Kidney	
Green	Estuary	H	145500	Y	Y	Y	?	1689
Green	Estuary	H	145501	Y	Y	Y	Y	2537
Green	Estuary	H	145502	Y	Y	Y	Y	568
Green	Estuary	H	145503	Y	Y	Y	Y	36
Green	Estuary	H	145512	Y	Y	Y	Y	291
Green	Estuary	H	145513	Y	Y	Y	Y	141
Green	Estuary	W	145541	Y	Y	Y	Y	2
Green	Estuary	W	145535	Y	Y	Y	Y	7
Green	Estuary	W	145551	Y	Y	Y	Y	46
Green	Estuary	W	145534	Y	Y	Y	Y	0
Green	Estuary	W	145547	Y	Y	Y	Y	919
Green	Estuary	W	145526	Y	Y	Y	Y	6
Green	Estuary	W	145527	Y	Y	Y	Y	7
Green	Estuary	W	145525	Y	Y	Y	Y	105
Green	Estuary	W	145522	Y	Y	Y	Y	13
Green	Estuary	W	145546	Y	Y	Y	Y	0
Green	Estuary	W	145548	Y	Y	Y	Y	1655
Green	Estuary	W	145550	Y	Y	Y	Y	0
Green	Estuary	W	145524	Y	Y	Y	Y	4330
Green	Estuary	W	145533	Y	Y	Y	Y	418
Green	Estuary	W	145545	N	N	N	N	759
Green	Estuary	W	145542	Y	Y	Y	Y	1975
Green	Estuary	W	145539	Y	Y	Y	Y	127
Green	Estuary	W	145536	Y	Y	Y	Y	40
Green	Estuary	W	145529	Y	Y	Y	Y	32
Green	Estuary	W	145530	Y	Y	Y	Y	5
Green	Estuary	W	145537	Y	Y	Y	Y	16
Green	Estuary	W	145549	Y	Y	Y	Y	368
Green	Estuary	W	145544	N	N	N	N	175
Green	Estuary	W	145538	Y	Y	Y	Y	1775
Green	Estuary	W	145540	Y	Y	Y	Y	3982
Green	Estuary	W	145543	Y	Y	Y	Y	2265
Green	Estuary	W	145521	Y	Y	Y	Y	2328
Green	Estuary	W	145523	Y	Y	Y	Y	0
Green	Estuary	W	145531	Y	Y	Y	Y	1602
Green	Estuary	W	145532	Y	Y	Y	Y	1304
Green	Offshore	H	145953	N	N	N	N	538
Green	Offshore	H	145955	N	N	N	N	1154
Green	Offshore	H	145956	N	N	N	N	8
Green	Offshore	H	145953	N	N	N	N	538
Green	Offshore	H	145955	N	N	N	N	1154
Green	Offshore	H	145956	N	N	N	N	8
Green	Offshore	W	145957	N	N	N	N	83
Green	Offshore	W	145958	N	N	N	N	16

Green Offshore W 145957 N N N N 83

River	Location	Origin	Fish ID	Histology				Total Nano cysts
				Gill	Liver	Heart	Kidney	
Green	Offshore	W	145958	N	N	N	N	16
Green	Offshore	H	145365	Y	Y	Y	Y	77
Green	Offshore	H	145369	Y	Y	Y	Y	5105
Green	Offshore	H	145365	Y	Y	Y	Y	77
Green	Offshore	H	145369	Y	Y	Y	Y	5105
Green	Offshore	W	145357	Y	Y	Y	Y	67
Green	Offshore	W	145366	Y	Y	Y	Y	Y
Green	Offshore	W	145367	Y	Y	Y	Y	488
Green	Offshore	W	145368	Y	Y	Y	Y	6
Green	Offshore	W	145357	Y	Y	Y	Y	67
Green	Offshore	W	145367	Y	Y	Y	Y	488
Green	Offshore	W	145368	Y	Y	Y	Y	6
Nisqually	Trap	W	145781	N	N	N	N	2811
Nisqually	Trap	W	145782	N	N	N	N	1871
Nisqually	Trap	W	145783	N	N	N	N	2
Nisqually	Trap	W	145784	N	N	N	N	250
Nisqually	Trap	W	145785	N	N	N	N	173
Nisqually	Trap	W	145786	N	N	N	N	2421
Nisqually	Trap	W	145787	N	N	N	N	2854
Nisqually	Trap	W	145788	N	N	N	N	3809
Nisqually	Trap	W	145789	N	N	N	N	1817
Nisqually	Trap	W	145790	N	N	N	N	2080
Nisqually	Trap	W	145669	Y	Y	Y	Y	87
Nisqually	Trap	W	145667	Y	Y	Y	Y	6552
Nisqually	Trap	W	145676	Y	Y	Y	Y	6535
Nisqually	Trap	W	145681	Y	Y	Y	Y	809
Nisqually	Trap	W	145682	Y	Y	Y	Y	322
Nisqually	Trap	W	145662	Y	Y	Y	Y	552
Nisqually	Trap	W	145666	Y	Y	Y	Y	14
Nisqually	Trap	W	145674	Y	Y	Y	Y	127
Nisqually	Trap	W	145672	Y	Y	Y	Y	0
Nisqually	Trap	W	145665	Y	Y	Y	Y	1871
Nisqually	Trap	W	145675	Y	Y	Y	Y	201
Nisqually	Trap	W	145678	Y	Y	Y	Y	213
Nisqually	Trap	W	145683	Y	Y	Y	Y	1108
Nisqually	Trap	W	145684	Y	Y	Y	Y	730
Nisqually	Trap	W	145685	Y	Y	Y	Y	1058
Nisqually	Trap	W	145686	Y	Y	Y	Y	7999
Nisqually	Trap	W	145687	Y	Y	Y	Y	895
Nisqually	Trap	W	145690	Y	Y	Y	Y	1747
Nisqually	Trap	W	145688	Y	Y	Y	Y	511
Nisqually	Trap	W	145689	Y	Y	Y	Y	450
Nisqually	Trap	W	145663	Y	Y	Y	Y	823
Nisqually	Trap	W	145664	Y	Y	Y	Y	1244
Nisqually	Trap	W	145668	Y	Y	Y	?	3065
Nisqually	Trap	W	145661	Y	Y	Y	Y	5626
Nisqually	Trap	W	145677	Y	Y	Y	Y	3302
Nisqually	Trap	W	145671	Y	Y	Y	Y	2104
Nisqually	Trap	W	145670	Y	Y	Y	?	514
Nisqually	Trap	W	145680	Y	Y	Y	Y	1296
Nisqually	Trap	W	145673	Y	Y	Y	Y	1246
Nisqually	Trap	W	145679	Y	Y	Y	Y	1031
Nisqually	Estuary	W	145735	Y	Y	Y	Y	97

Nisqually	Estuary	W	145737	Y	Y	Y	Y	2023
Nisqually	Estuary	W	145738	Y	Y	Y	Y	4138
Nisqually	Estuary	W	145739	Y	Y	Y	Y	960
Nisqually	Estuary	W	145740	Y	Y	Y	Y	1106
Nisqually	Estuary	W	145741	Y	Y	Y	Y	446
Nisqually	Estuary	W	145742	Y	Y	Y	Y	2111
Nisqually	Estuary	W	145743	Y	Y	Y	Y	6152
Nisqually	Estuary	W	145744	Y	Y	Y	Y	1535
Nisqually	Estuary	W	145745	Y	Y	Y	?	4106
Nisqually	Estuary	W	145747	Y	Y	Y	Y	3382
Nisqually	Estuary	W	145749	Y	Y	Y	Y	3764
Nisqually	Estuary	W	145736	Y	Y	Y	Y	1505
Nisqually	Estuary	W	145746	Y	Y	Y	Y	660
Nisqually	Estuary	W	145748	Y	Y	Y	Y	440

River	Location	Origin	Fish ID	Histology				Total Nano cysts
				Gill	Liver	Heart	Kidney	
Nisqually	Estuary	W	145732	Y	Y	Y	Y	6405
Nisqually	Estuary	W	145731	Y	Y	Y	Y	1683
Nisqually	Estuary	W	145733	Y	Y	Y	Y	2364
Nisqually	Estuary	W	145734	Y	Y	Y	Y	4157
Nisqually	Estuary	W	145721	Y	Y	Y	Y	4456
Nisqually	Estuary	W	145722	Y	Y	Y	Y	4377
Nisqually	Estuary	W	145723	Y	Y	Y	Y	104
Nisqually	Estuary	W	145724	Y	Y	Y	Y	10
Nisqually	Estuary	W	145725	Y	Y	Y	Y	9844
Nisqually	Estuary	W	145726	Y	Y	Y	Y	2692
Nisqually	Estuary	W	145727	Y	Y	Y	Y	1640
Nisqually	Estuary	W	145728	Y	Y	Y	Y	1646
Nisqually	Estuary	W	145729	Y	Y	Y	Y	1246
Nisqually	Estuary	W	145730	Y	Y	Y	Y	744
Nisqually	Offshore	W	145751	Y	Y	Y	Y	698
Nisqually	Offshore	W	145752	Y		Y	Y	0
Nisqually	Offshore	W	145753	Y	Y	Y	Y	3245
Nisqually	Offshore	W	145754	N	N	N	N	401
Nisqually	Offshore	W	145751	Y	Y	Y	Y	698
Nisqually	Offshore	W	145752	Y	Y	Y	Y	0
Nisqually	Offshore	W	145753	Y	Y	Y	Y	3245
Nisqually	Offshore	W	145754	N	N	N	N	401

Study 8: Hatchery Coho Telemetry Study

Principal Investigator = Megan Moore (NOAA Fisheries)

Overview

Telemetry studies of steelhead (*Oncorhynchus mykiss*) smolts in the Puget Sound have indicated that approximately 80% of fish entering marine waters do not survive to the Pacific Ocean (Moore et al., in review). Telemetry data for coho in South Puget Sound also suggest high early marine mortality (unpublished S. Steltzner, Squaxin Tribe), and the long-term declines in smolt-to-adult survival for Puget Sound steelhead (unpublished Kendall, WDFW) and coho (Zimmerman 2015) have been similar, suggesting that there may be a common source of mortality.

Analyses of survival patterns have revealed that outmigration timing may influence the survival success of steelhead smolts migrating from river mouth to the Pacific Ocean. For example, Moore et al. estimated low survival rates of juvenile steelhead migrating through Puget Sound during the first week of May for several Puget Sound populations during each of four study years (2006-2009), in relation to higher survival rates in late April and late May. In 2014, survival rates of smolts from the Nisqually River declined linearly with release date from late April to late May (Megan Moore, unpublished data). One possible factor that may be driving these temporal patterns is the release of large numbers of hatchery coho salmon (*Oncorhynchus kisutch*) smolts. Large numbers of prey moving through South Puget Sound together may be attracting predators to the foraging area (aggregation response, see Wood 1985), increasing the mortality of coho and co-migrating steelhead that may otherwise survive better in more dispersed outmigration groups.

Objectives

The proposed study would generate data on migration timing, abundance patterns, and mortality distribution of hatchery coho salmon smolts throughout Puget Sound. With this information we would be able to assess peak coho migration timing and compare temporal and spatial mortality patterns of steelhead and coho smolts, allowing us to identify whether mortality increases with juvenile coho abundance in South Puget Sound, and whether similar mortality sources (i.e. harbor seals) are affecting both populations.

H_{01}: Peak timing of coho migration coincides with periods of low steelhead smolt survival

H_{02}: Rates and spatial patterns of coho mortality are similar to those of steelhead smolts

Study design

Study Population

Yearling hatchery coho smolts will be obtained from South Sound Net Pens, Garrison/Deschutes, Minter Creek, or Squaxin Net Pens. Vemco V7 acoustic transmitters (69 kHz, 7 mm diameter, 20 mm length, 1.6 g) will be surgically implanted in 100 hatchery smolts as outlined in Moore et al. (2010). Only smolts weighing greater than 25 g will be tagged to maintain a tag to body weight ratio of less than 6.5%.

Smolts will be held overnight and released with the rest of their cohort.

Data Collection

Receiver deployment: Tags will be detected at four Vemco VR3 receiver arrays: near the Tacoma Narrows (8 receivers; NAR), in Central Puget Sound (19 receivers; CPS), in Admiralty Inlet (13 receivers; ADM), and at the western end of the Strait of Juan de Fuca (30 receivers; JDF; maintained by the Ocean Tracking Network; Moore et al. in review; Fig. 1).

Mobile Tracking: The proposed study will take advantage of mobile tracking activities scheduled for after the coho and steelhead migration. A mobile (deployed from a boat) receiver will be used to "listen" for "dead" steelhead and coho tags (continuously pinging in one location, assumed to have been eaten and deposited by a predator) along the smolts' migratory route.

Seal Packs: The proposed study will also take advantage of the 12-18 harbor seals that will have acoustic receiver – gps tracking packs attached to their backs (See study 2). The seals will help determine the number of tags deposited near seal haulouts, a sign that the associated fish was eaten by a seal. These data will be used to help establish a predation rate.

Data Analysis

Detection data will be used to populate mark-recapture models, which will be used to estimate the survival rates of coho smolts through each migration segment (release to EST, EST-NAR, NAR-CPS, CPS-ADM, and ADM-JDF). A 'species' factor can be included in models utilizing both steelhead and coho detection data for a direct comparison of survival patterns. Migration behavior metrics such as travel time and residence time in each segment will be calculated using the time of detection at each receiver array. Any dead coho tags located during mobile tracking will be plotted on a map alongside steelhead mortalities for comparison.

Outcomes

This telemetry study will provide a detailed comparison of the survival and migration patterns of coho salmon and steelhead smolts in the Nisqually River. We will be able to test whether hatchery coho migration timing and duration coincide with periods of lower steelhead survival. We will also be able to determine whether spatial mortality patterns are similar between species, which may suggests a common predator(s) or other mortality source. Comparison of migration behaviors between species will help us understand the degree of overlap in habitat utilization and whether steelhead mortality throughout the migration route may be affected by interactions with hatchery coho. Ultimate outcomes may include actions to directly manage the mortality source, and/or better disperse hatchery releases in space and time, to reduce a predator "call to the table" and increase the survival of hatchery coho and wild steelhead.

Time Line

Activity	Start Date
Finalize receiver array design	December 2015
Purchase receivers, tags, and tagging supplies	January 2016
Deploy receiver arrays	March 2016

Tag and release steelhead smolts	April-June 2016
Retrieve and download receivers	August 2016
Data analysis	September 2016
Reporting	January 2017

Deliverables

Results of the telemetry study will be summarized and submitted for publication to a peer-reviewed scientific journal by July 2017. Data will also be presented orally to interested parties and at relevant scientific meetings.

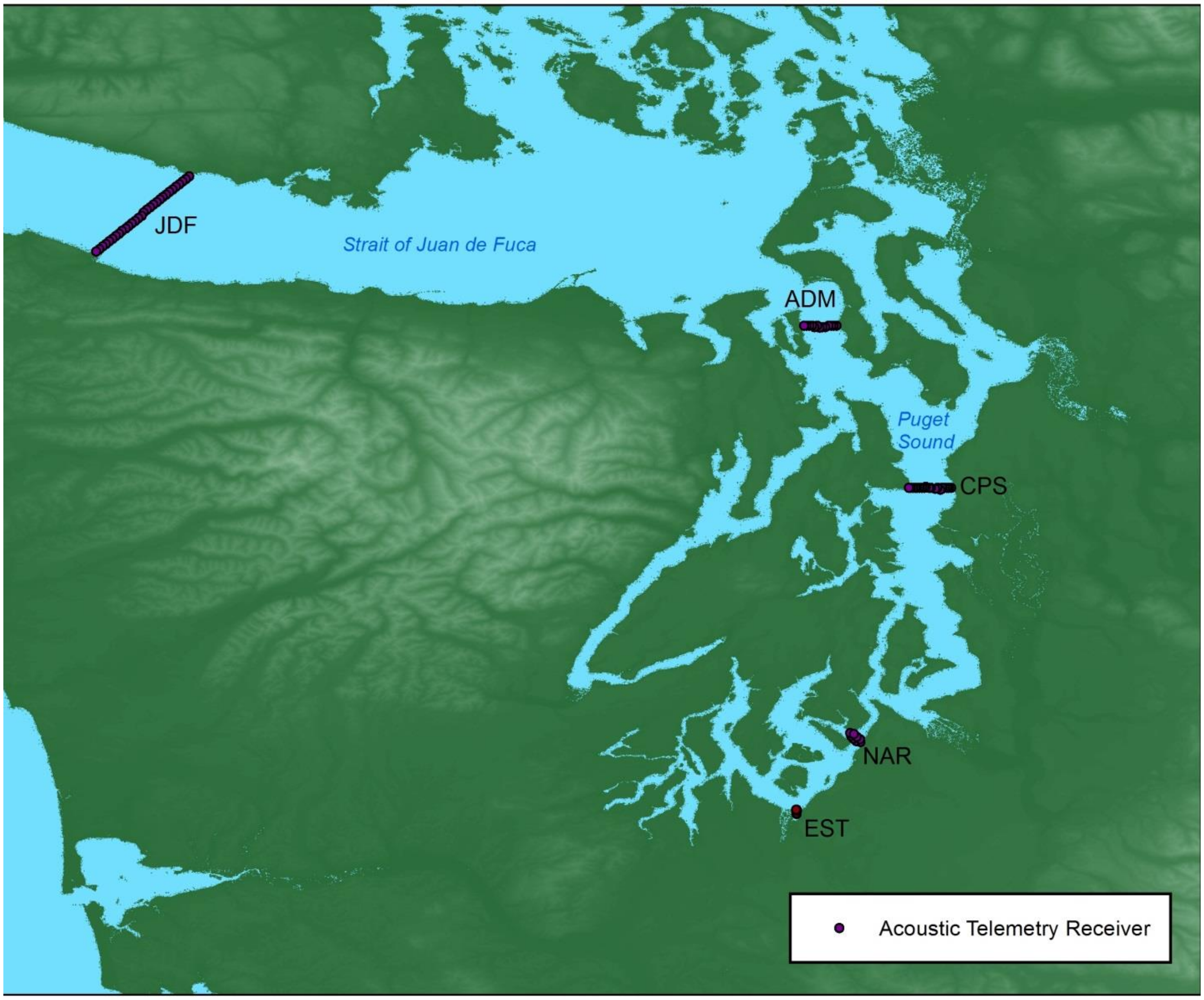
References

Moore ME, Berejikian BA, Goetz FA, Berger AG, Hodgson SH, Conner EJ, Quinn TA (in review) Multi-population analysis of Puget Sound steelhead survival and migration behavior. Marine Ecology Progress Series.

Collis K, Beaty RE, Crain BR (1995) Changes in catch rate and diet of northern squawfish associated with the release of hatchery-reared juvenile salmonids in the Columbia River reservoir. N Am J Fish Manage 15:346-357.

Wood CC (1985) Aggregative response of common mergansers (*Mergus merganser*): predicting flock size and abundance on Vancouver Island salmon streams. Can J Fish Aquat Sci 42:1259-1271.

Figure 1. Map of study area showing acoustic telemetry receiver arrays in the Nisqually river estuary (EST), south of the Tacoma Narrows (NAR), in Central Puget Sound (CPS), in Admiralty Inlet (ADM), and spanning the Strait of Juan de Fuca (JDF).



Study 9: Lipid content analysis and next steps with contaminants

Principal Investigator = Sandie O'Neill (Washington Department of Fish and Wildlife)

The lipids of 60 individual steelhead samples collected in 2014 will be analyzed: % lipids as well as composition of lipid classes. The detailed lipid results will be compared to the intensity of the *Nanophyetus* parasite in individual fish to see if they are correlated. Secondarily, chemical analyses of juvenile steelhead collected in 2015 at the Nisqually River smolt trap will be repeated (as was done in 2014) to determine if PBDE contamination is a consistent problem in this river system. These PBDE results will then be used to determine if additional PBDE studies are needed in 2016.

APPENDIX B: INFORMATION SUPPORTING WORKGROUP DECISION MAKING

The following documents were used…

Table 1. Studies/Research components discussed with considerations of the Workgroup

Objectives	Key study objective	Study Components	Considerations
Note: On September 1, the predation group concluded that both the telemetry and seal scat work were of value, complementary, and may serve to validate one another. They also concluded a dinner bell retest is important. Both of the proposed approaches would utilize predation rate modeling (separately or in combination) customized by Eric Ward for the type data collected.			
A,B,D, H,O,P	Estimate a predation rate by harbor seals on steelhead in Puget Sound (south of Admiralty Inlet), and determine whether predation by harbor seals differs by region	Tag tracking and seal behavior – Tag 200 Nisqually steelhead (150 V7 and 50 V9 Temperature Tags) & 18 seals. Develop enhanced array and employ mobile tracking. Quantify seal time at depth and locations to estimates time seals spend at haulouts (to assess probability of tag defecated near a haulout site). Incorporate harbor seal abundance, behavior, steelhead tag locations, and smolt abundance to estimate the predation rate and total # of smolts consumed by seals. [**Barry B.,** Megan M., and Steve J.]	Potential outcomes: 1) Can estimate how many fish survive and determine where a substantial percentage of the non-survivors end up (using combined tag detection approaches). These data can be expanded based on harbor seal population estimates and behavior. 2) All data are certain to be reflective of a target population (Nisqually River population) 3) Broad spatial coverage throughout the main basin of Puget Sound by tagging north, central, and south PS harbor seals, mobile tracking, and fixed arrays in all areas 4) Detailed movement patterns of tags provide behavioral indicators of predation. 5) Can infer the fate of a high percentage of tagged fish (via combo of harbor seal tags, mobile tracking, & fixed receiver arrays). Can combine with 2014 data to make more robust estimate of predation rates. 6) Demonstrated approach in 2014 and 2015 that tags and their locations can be readily identified. 7) Temperature tags could provide additional evidence of source of predation. Although, they aren't archival so limited to data collected while tag is ingested by predator. 8) Continues to provide information on mechanisms such as body size, migration timing, migration route, estuarine behavior, and indications of prey switching (if combined with tagging of hatchery coho) Limitations: 1) Predation events are inferred from tag behavior and final locations. There may be other predators using haulouts. 2) Tags may affect vulnerability to predation, regardless of whether the sound is important (e.g., tag burden). However, there have been no signs of size-selective mortality in steelhead tagged over several years of telemetry work. 3) If there is a tag effect (related to noise or not), we don't know whether the effect is stronger for different potential predators (e.g., seals and harbor porpoise). 4) Study is focused specifically on one steelhead population which limits ability to expand results to entirety of Puget Sound.

A,B,P	(cont'd) Estimate a predation rate by harbor seals on steelhead in Puget Sound (south of Admiralty Inlet), and determine whether predation by harbor seals differs by region	**Seal scat analysis – South (and potentially Central) Puget Sound** - Analyze scat. Quantify steelhead in diet and use to estimate predation rate. Diet composition of seals before and during outmigrant time period. Collect 70 scats from each quadrant every two weeks, mid- March through end of June. Due, at a minimum (1) South Puget Sound quadrant affiliated with Nisqually steelhead pop, and max also include (2) Central Puget Sound and (3) Admiralty: 2 = the quadrant between Tacoma Narrows line to Central Puget Sound telemetry line, and 3= Central Puget Sound line to Admiralty line. May combine 2 and 3 though due to potential limitation in # of scats that can be collected from each quadrant. [**Austen T.,** Steve J., Scott P., Ken W., Monique Lance) and Nisqually Tribe support]	Potential outcomes: 1) Provides direct evidence of predation. That is, DNA in the scat samples can be confidently identified to species. 2) New methods allow for estimating percent diet composition by species consumed. 3) Secondary benefit. While not an objective of this Workgroup's effort, it provides information on predation of other salmon species of concern (esp. juvenile coho and Chinook). Limitations: 1) Very high predation rates may only be reflected as a very small (1%) of the harbor seal diet as described in worksheet distributed. Concern is that the absence of steelhead in the diet may represent a false negative, or that confidence in a resulting predation rate estimate may be limited as a result of small proportions in diet. 2) The number of juvenile coho in the system is larger compared to that of steelhead, which may impact resolution. However, there is confidence that hard parts for diff species of juveniles may be distinguishable, combined w/ quantification from DNA. 3) Approach for estimating % of steelhead consumed based upon a fair number of assumptions & intermediate calculations. 4) Due to mixing of steelhead populations and variable data on steelhead population outmigrant abundance, it is increasingly difficult to assign a predation rate as you move north through Puget Sound. Highest confidence for South Puget Sound haulouts/predation of Nisqually steelhead. Central Puget Sound quadrant (adding Puyallup & Green pops abundance) may also be feasible. 5) Only yields a response for the colony/haulout sites sampled. 6) Sampling sites may not reflect vulnerability to predation depending on the availability of smolts (e.g., Cutts island seals are very unlikely to encounter steelhead). So the data are specific to a particular haulout location but not to a particular steelhead population(s), which is the focus. 7) To fully estimate predation rate for Nisqually steelhead pop, scat samples would need to be collected throughout Puget Sound to get at a predation rate because we know from the telemetry data that steelhead are dying and ending up at haulouts from south PS to Admiralty Inlet.
A,B		**Seal abundance assessment** - Aerial survey. Estimate seal abundance during steelhead outmigration period Range = Pt. Wilson to Olympia [**Steve J**]	• Required for estimating predation rate by seals (for both studies, above). Standard approach used for decades. • May underestimate population size due to capacity of haulout sites (estimates pops by abundance hauled out, not in water)
D,P?		**Dinner bell** – Higher power assessment of potential for dinner bell effect. Either 150 Nisqually steelhead w/ delayed tags combined with results from 2014, or some other, but more costly approach. [**Barry B.,** Megan M.]	• Workgroup must weigh what level of power they are comfortable with attaining vs. cost and feasibility. • Predation folks (Sept 1) determined best to be able to detect a 10% absolute difference in juvenile steelhead outmigrant survival from release to the SJDF/Admiralty combined arrays. Eric W. concluded that sample sizes need to be 250 per group, if using survival and detection rates of 2014 for Nisqually. Eric and Barry are revisiting given increased probability of detection in 2016. • While power is limited, current evidence suggests no difference in mortality rates between pinging and non-pinging tags (based upon detections at SJDF/Admiralty arrays and seal haulouts). Other studies suggest tag effect, but include 2 components (a motivated predator and the opportunity for predators to learn the association). Also, the most relevant study, in the Columbia River, focused on adult Chinook and was below a migration obstruction, Bonneville Dam. • Nisqually steelhead best source; however, concerns about using an additional 150 fish due to impacts on smolt trap efficiency/mark-recapture program. And, 150 combined with previous year will not reach recommended sample size to detect 10% absolute difference in survival (see bullet 1). Could add a delayed tag group to *Nanophyetus* study and increase group sizes; however, concerns about confounding effects reducing power/effective sample size (hatchery fish and release location)

O,P	Determine whether timing of coho hatchery releases influence steelhead survival by attracting predators	**Coho hatchery releases** - Assess whether peak timing of coho migration coincides with periods of low steelhead smolt survival; and whether mortality rates and spatial migration (and mortality) patterns of coho are similar to those of steelhead smolts. Tag 100 hatchery coho. [**Megan M.,** Barry B.] *On September 1, determined that scat analysis was likely too infrequent to detect signal. Concluded that telemetry is best shot.*	• Telemetry provides the greatest opportunity for capturing a temporal impact that could be occurring within a two-week window. • Choosing a relevant coho population is necessary. Most likely the South Sound Net Pen release of 1.1M coho that occurs in May and not the Kalama (Nisqually) Hatchery release that occurs in April. However, could just be a delayed effect that is triggered by April releases combined with steelhead outmigrants. • Secondary benefit. While not an objective of this Workgroup's effort, it provides information on predation of juvenile coho, another salmon species of concern.
H	Determine direct and indirect effects of *Nanophyetus*	Assess differences in survival/fate of steelhead with and without *Nanophyetus.* Release up to 100 infected and 100 nano free steelhead. Lab test to assess swimming performance and direct mortality [**Paul H.,** Martin C., Barry B., Megan M.]	• Will determine whether Nanophyetus is contributing to or the root cause of steelhead mortality (direct or predation-based). • May also help quantify the contribution to predation-based mortality to extrapolate impacts to nano vs nano-free steelhead populations • Release location not yet identified. However, release must occur in saltwater out of watersheds where they could be exposed to *Nanophyetus*. Also, release location should be in area where steelhead are still likely to experience substantial predation-based mortality (e.g., South Puget Sound). • Like dinner bell, investigators must determine what difference in survival they would like to be able to detect. Current sample size of 100 infected vs 100 nano free. This may be insufficient and the effective sample size may be reduced by confounding effects associated with the source population (hatchery fish) and release location.
I	Develop Nano qPCR, determine timing /seasonality of *Nanophyetus* shedding events, identify *Nanophyetus* hot spots	Use qPCR developed by USGS to identify peak shedding events and prevalence and intensity of *Nano* in juga snails within and between watersheds [**Paul H.,** Martin C., Bruce S.]	• Helps determine whether we can manage "around" peak shedding events (rearing locations & release timing), at a minimum for hatchery fish. • Helps isolate hotspots for managing snail populations in particular watersheds. • Dependent upon whether additional funding will be received internally by USGS Western Fisheries Research Center
E,N	Complete retrospective assessment of SAR data and correlations with fish and environmental characteristics	Use SAR data compiled in 2013-2015 and build upon fish characteristic and environmental data to complete this assessment. Dig into findings of more significant correlations [**Neala K.**]	• Provides some of the greater potential to help explain changes over time, to put current findings in context of broader ecosystem context, and to build connections among the factors at play. • Data will contribute to ecosystem indicators and ecosystem modeling efforts that are part of the greater Salish Sea Marine Survival Project. • Work limited by data available. • Much of the data are coarse.
J,K	Increase power of GWAS and investigate relationship w/ Nano infections	Proposal to add 2015 Nisqually samples (100 acoustic tagged) to the existing GWAS study to increase the power of results. Secondarily, proposal to use DNA samples collected in 2014 in follow-up GWAS to assess potential relationship between loci affiliated with mortality in initial GWAS and nano cyst count and heart and gill inflammation [**Ken W.**]	• The Workgroup determined that it was critical to increase the power of the results. This work would help. • Questions regarding whether the *Nanophyetus* correlations should be done "after" the proposed *Nanophyetus* control/treatment experiment is done, to resolve whether or not Nanophyetus is contributing to mortality first. • Ken W. also recommended assessing the microbiome (e.g. film on steelhead skin) as exploratory research.

M	Better assess whole body lipid content and condition factor relative to *Nanophyetus* loads and survival of outmigrating steelhead	Assess 60 individual steelhead samples from 2014 study. Will also consider inclusion of individual samples from 2015 Nisqually captures. **[Sandie O.]**	• Sandie is continuing this work in attempt to resolve whether or not whole body lipid content and condition factor are a concern. This work is being paid for by WDFW water quality group. • Literature review performed by J. McMillan (Trout Unlimited) would suggest lipid levels seen (less than 1%) were not inconsistent with a decline in whole body lipid content toward depletion during the smolt life-stage.
L	Follow-up to PBDE results in Nisqually	Do chemical analyses on the 2015 Nisqually steelhead samples to ascertain if the PBDEs contamination is a consistent problem in this river system.	• Will need to ascertain whether PBDE results were driven by estuary or trap samples in 2014. If estuary samples, trap samples from 2015 will not be a direct comparison. • The PBDE results from the 2015 Nisqually steelhead samples will used to decide if additional studies are needed in 2016. • This work is being paid for by WDFW water quality group.
	Deemed not feasible, not the appropriate time, or to be dealt with via other processes		
C	Assess impact of other potential predators	On September 1 the group that met to discuss predation did not come up with approaches for other predators of concern. Instead, concluded they should continue to be investigated, but a great deal can still be obtained from the focused work on harbor seals. Also, Scott P. recommended general observations of other potential predators co-occupying haulouts (e.g cormorants) should be performed. Following the meeting, Steve J. and others also concluded that learning the results of the recent study commissioned by the Navy to assess harbor porpoise abundance and distribution would be a good first step for further considerations of assessing harbor porpoise impacts. LLTK has also flagged assessing harbor porpoise impacts as an area of interest in requests to outside funders.	
G, but could contribute to other objectives	Compare contrasting systems with different survival patterns	Compare Hood Canal and the Skokomish steelhead population to Puget Sound and the Nisqually steelhead population. Activities may include tag tracking, seal behavior, seal diets, prey availability comparisons, and correlations and ecosystem modeling. **[Lead not identified,** Megan M., Barry B., Steve J., Scott P. , etc.]	**Rationale** – *Primarily trying to find some way to determine whether rapid outmigration is associated with food limitations, and whether this migration behavior increases susceptibility to predation*. Higher numbers of seals and yet lower mortality in Hood Canal compared to Puget Sound, up until the HC Bridge. Slower migration rate in Hood Canal vs Puget Sound. Why? Potentially more food available to steelhead in Hood Canal, more alternative food sources for seals, lower *Nano* loads? The Workgroup did not proceed largely because the Hood Canal Bridge Impact Assessment (2017) will not happen in the same year as the Puget Sound steelhead work (2016). However, there may be opportunities to compare results across years, and to compare new zooplankton data across regions to determine whether there may be support for the food limitation>foraging behavior>increased predation hypothesis. **NOTE: Diets of steelhead collected in 2014 throughout Puget Sound, and Nisqually steelhead collected in 2015 will be assessed for gut content and prey types as a preliminary effort to determine whether foraging should be concern.**
Q	Model South Puget Sound ecosystem to combine drivers and build out to broader ecosystem effects	Build upon existing South Sound EwE model (OR DO IN NEW ATLANTIS MODEL) to better incorporate changes in forage fish production and bottom-up shifts along with seal (and other) predation (and potentially Nano data) to try and better illustrate the magnitude of combined effects, and steelhead pop sensitivity to change. However, steelhead such small part of food web, it may be difficult to make heads/tails of results. [**Chris Harvey,** Isaac Kaplan, Dave Preikshot, or post-doc]	Puget Sound ecosystem modeling will begin soon via the greater Salish Sea Marine Survival Project. An initial focus on South Puget Sound, and/or comparing South Puget Sound to another contrasting basin, will be considered as an initial activity in the process of establishing an end-to-end ecosystem model for Puget Sound.
	Discussed but not part of priorities		
	Identify predator specialists	**Rationale -** Likely not worth trying given the amount of feces needed to discriminate specialists. Next round of work will help hone in on seal populations to focus on for a specialist assessment.	

	Determine if seal behavior changes with steelhead outmigrant timing and hatchery releases	**Rationale –** No overtly apparent changes in 2014 seal behavior before vs. after steelhead peak outmigration, or hatchery releases. Therefore, may not be worth effort.
	Assess disease-predation rate correlation via seal scat analysis	Look at prevalence of specific diseases in seal scat that includes steelhead. **Rationale** - Concern is that prevalence of certain diseases in juvenile steelhead (like Nano) is so high that we wouldn't be able to distinguish whether or not presence = higher likelihood of being eaten. Would need to be able to characterize intensity of infection by consumed individual steelhead. Also, may be difficult to attribute specific diseases to specific species in scat?
	Improving SARs	PIT tagging for more robust analysis of SARs. Due to timeframe to implement and get results, this is outside of scope of work. *Will be recommendation in report (from Kendall work) for better handle of SARs*

Table 2. Workgroup study ranking

Steelhead Marine Survival Workgroup - 2015-2017 Study Ranking Exercise

Item	Study (or study component)	1	2	3	4	5	6	7	8	9	10	11	12	13	Avg	Rank	Notes
B	Seal predation - scat/diet analysis (South Puget Sound quadrant only)	2	4	3	4	3	1	6	2	7	3.5	2	6	7	3.9	**1.0**	1. Critical to acquire direct evidence for seal predation | 2. Averaged 3-5 = 4. If we want to do the model, we need all 3 areas. With only Area 1, we will be limited to analysis and modelling for Nisqually | 3. | 4. | 5. An estimate of seal predation that is direct, and independent of telemetry bias would strongly reinforce findings of earlier work. I am confident sample size can be attained here. | 6. | 7. | 8. | 9. | 10. |11. Need to clearly identify predator per Item A. |12. |13. complete analysis in Central Sound first to see if there is any evidence of predation
A	Seal predation, etc- telemetry analysis	8	2	1.5	3	4	5	1	1	14	1	1	7	4	4.0	**2.0**	1. Extremely rich information, but any way you cut it, interference to the mortality agent (i.e., predator) is indirect and based on location. Suggest considering a study design that prioritizes more stationary receivers near seal haulouts, cormorant roosts etc. but no seal tagging. In my opinion, this could be more informative than tagging seals. | 2. Essential to completing dinner bell testing and improve understanding of harbor seals | 3. | 4. | 5. Better monitoring of haulout sites very useful for estimating predation, and subsequent data on survival really important for interannual variability | 6. Would be nice to do seal telemetry, but not necessary in my opinion, especially when considering price. Seems we can find out what we need to with fixed receivers and mobile tracking | 7. | 8. | 9. | 10. |11. Predation appears to major reason for steelhead mortality in Puget Sound, with disease a contributing factor. Need to better identify where predation is taking place over large area.|12. |13.
E	Seal predation rate - aerial survey for seal abundance estimate (applies to A-D, above)	3	6	1.5	5	2	4	7	3	9	3.5	5	10	11	5.4	**3.0**	1. Need to know total seal abundance in order to estimate consumption of steelhead | 2. 240 hours for salary and benefit cost for the aerial survey task need to come out of new RS1 position listed in Items B, C and D. | 3. | 4. | 5. This is essential for any predation rate estimate, even if we chose not to collect additional seal data we could use these counts with 2014 data to estimate predation rate from stationary tags. | 6. | 7. | 8. | 9. | 10. |11. Need to better identify spatial distribution of seals, but only required if Items A-D show that seals are key steelhead predators in Puget Sound.|12. |13. defer until after analyses of scat samples
C	Seal predation - scat/diet analysis (add Central Puget Sound quadrant)*	4	4	6	9	9	2	8	6	8	3.5	3	8	2	5.6	**4.0**	1. Estimating total consumption of steelhead by seals is very valuable information despite uncertainty over total number of steelhead to enter Puget Sound marine environment. | 2. Could combine Central Puget Sound with Admiralty and reduce DNA analysis cost by 1/3. Combined quadrants could info for modelling. | 3. | 4. | 5. Important area to understand, but reduced ability to compare to total smolt abundance | 6. | 7. | 8. | 9. | 10. |11. Need to clearly identify predator per Item A. |12. |13.
H	Determine direct and indirect effects of Nanophyetus infections	7	13	4	1	5	7	2	4	11	6.5	6	3	3	5.6	**4.0**	1. Rate and magnitude of Nanophyetus infection appears to correlate with survival rates (i.e., poor survival south sound). Important to evaluate the ultimate impact of the pathogen. | 2. Averaged Ranks 12,13, 14 = 13. Disease and Nano tasks | 3. | 4. | 5. I am still not convinced this is the only pathogen worth investigating, but a proper assessment of the impacts of infection on survival probability seems worth doing for sure. | 6. | 7. | 8. | 9. | 10. |11. Nanophyetus infections may increase susceptibility to predation by steelhead smolts. This hypothesis supported by studies in Oregon, and well as higher predation rates on otherwise high condition steelhead smolts in south sound.|12. My focus is organismal and resolving freshwater/health/condition questions|13.
L	Complete retrospective assessment of SAR data and correlations with fish and environmental characteristics	1	7	10	8	1	6	3	10	2	9	9	11	1	6.0	**6.0**	1. Only study addressing total lifetime marine mortality | 2. | 3. | 4. | 5. Given than the logic of the project is based on a difference in survival between two time periods, a complete retrospective analysis of factors should be highest priority. | 6. Inexpensive and important for big picture | 7. | 8. | 9. | 10. |11. Retrospective analysis of environmental conditions needs to be completed to determine if increased predator numbers, and increased predation rates on steelhead, can predict decline in steelhead smolt mortality after adjusting for large-scale environmental conditions and shifts in forage fish abundance.|12. |13.

F	Again test potential for dinner bell effect to increase power of assessment	9	1	8	6	8	10	5	5	1	11	12	1	14	7.0	**7.0**	1. Important to evaluate dinner bell hypothesis but we already have some information on this. Other studies ranked higher have greater chance of providing new information. | 2. Need to have more power to put this to rest. | 3. | 4. | 5. I was initially of the mind that this is important, but have since decided that there are many out there who will never be convinced there is no tag impact on predation. May be waste of money. | 6. Important but not the most important. If we can't tag lots of Nisqually smolts, I am hesitant about the value (cost + potential results given hatchery fish characteristics) of tagging Puyallup hatchery fish | 7. | 8. | 9. Absolutely critical. Our whole estimate of early marine survival and our VMTS data relies on there being no dinner bell effect. Two Statisticians have now told us that we need 250 fish in each sample. | 10. |11. |12. This needs to be resolved. Spread work over two outmigration years|13. not necessary; dinner bell effect does not explain overall decline in SARs
D	Seal predation rate - scat/diet analysis (add Admiralty quadrant)*	5	4	7	10	10	3	9	7	12	3.5	4	13	8	7.3	**8.0**	1. Estimating total consumption of steelhead by seals is very valuable information despite uncertainty over total number of steelhead to enter Puget Sound marine environment. | 2. | 3. | 4. | 5. Reduced ability to compare to total smolt abundance | 6. | 7. | 8. | 9. | 10. |11. Need to clearly identify predator per Item A. |12. |13.
I	Identify Nanophyetus hotspots and timing/seasonality of nano events w USGS qPCR	11	10.5	9	2	12	13	4	9	13	6.5	7	9	9	8.8	**9.0**	1. Suggest evaluating Nanophyetus impacts (study H) before adding this level of resolution | 2. Averaged Ranks 10 and 11 = 10.5 | 3. | 4. | 5. This could/should be a follow up study to item H. Only worth doing if nano is determined to be an issue. | 6. Can be done at a later time if Nano turns out to be an issue | 7. | 8. | 9. Seem like you would do the other Nano work and depending on the outcome do this work if there is a strong effect. | 10. |11. Complements Item H. |12. Don't the fish do this for us?|13.
J	Increase power of 2014 GWAS analysis	6	9	14	13	11	8	10	11	4	12	11	2	5	8.9	**10.0**	1. Important to keep broad perspective on possible mechanisms of mortality. | 2. | 3. | 4. | 5. This is great exploratory work, but also seems likely to produce a red herring. Mortality is expected to be related to genome, the question is if a change occurred in the population to increase mortality. | 6. | 7. | 8. | 9. | 10. |11. GWAS analysis implies that survival may be associated with genetically determined immunity levels. Need to establish that disease is contributing to increased mortality levels of smolts.|12. My focus is organismal and resolving freshwater/health/condition questions|13.
G	Determine influence of coho hatchery releases / pulse outmigrant abundance on steelhead survival	10	8	5	11	13	11	11	8	3	10	10	12	13	9.6	**11.0**	1. Interesting hypothesis but I think there are also other potential prey species/stocks that could account for prey switching (e.g., south sound net pen coho) so I am not convinced this will give a definitive conclusion | 2. | 3. | 4. | 5. I don't see how this study answers the question. Not clear how data produced will tell us about steelhead probability of survival…seems only loosely correlative. | 6. Concern that since we aren't assessing all coho in the region, a negative finding may not mean that coho don't matter and that the predators aren't prey switching | 7. | 8. | 9. Would like to see this as an experiment where timing is adjusted. If that were the case, I would rank this much higher. | 10. |11. Would be good to determine if juvenile steelhead SAR rates are correlated with coho hatchery releases, in addition to other environmental factors assessed in Item L. |12. |13.
K	Better assess whole body lipid content and condition factor relative to *Nanophyetus* loads and survival of outmigrating steelhead	12	10.5	12	7	7	12	12	14	10	8	8	5	12	10.0	**12.0**	1. Need to get a better understanding of Nanophyetus impacts (study H) before addressing lipid, condition, etc. | 2. Averaged Ranks 10 and 11 = 10.5 | 3. | 4. | 5. The nutritional status of these fish seems pretty important to understand completely, and potential interactive effects | 6. Doesn't seem as important as steelhead smolts are known to be low in lipids | 7. | 8. | 9. | 10. |11. Complements Item H. |12. My focus is organismal and resolving freshwater/health/condition questions|13. Study will be conducted with existing WDFW funds.
I	Assess potential relationship between loci affiliated with mortality in initial GWAS and nano cyst count and heart and gill inflammation	13	13	11	12	6	9	13	12	5	13.5	14	4	6	10.1	**13.0**	1. In my opinion, GWAS most informative for identifying possible mechanisms of mortality. Nanophyetus is already on our radar screen. | 2. Averaged Ranks 12,13, 14 = 13. Disease and Nano tasks | 3. | 4. | 5. If nano (or other pathogen) infection is important, an understanding of population susceptibility seems worthwhile. | 6. | 7. | 8. | 9. | 10. |11. Need to first show that predators are causing mortality, and then that differences in physiological condition and disease susceptibility is contributing to increased predation rates.|12. My focus is organismal and resolving freshwater/health/condition questions|13.

*see considerations worksheet for rationale for by-quadrant ranking for seal scat analysis