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SALISH SEA MARINE SURVIVAL PROJECT

Puget Sound Steelhead Marine Survival: 2013-2015 research findings summary

Puget Sound Steelhead Marine Survival Workgroup December 31, 2015

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Puget Sound Steelhead Marine Survival Workgroup (for 2013-2015 research phase)
Barry Berejikian, NOAA Northwest Fisheries Science Center*
Chris Ellings, Nisqually Indian Tribe*
Ed Connor, Seattle City Light
Erik Neatherlin, Washington Department of Fish and Wildlife *
Joseph Anderson, Washington Department of Fish and Wildlife
Ken Warheit, Washington Department of Fish and Wildlife *
Martin Chen, Northwest Indian Fisheries Commission
Megan Moore, NOAA Northwest Fisheries Science Center
Neala Kendall, Washington Department of Fish and Wildlife*
Paul Hershberger, US Geological Survey, Marrowstone Marine Field Station*
Sandra O'Neill, Washington Department of Fish and Wildlife*
Scott Pearson, Washington Department of Fish and Wildlife
Steve Jeffries, Washington Department of Fish and Wildlife

Contributing Experts: Field Work and Analysis
Adam Hansen, University of Washington
Andrew G. Berger, Puyallup Tribe
Andrea Carey, Washington Department of Fish and Wildlife
Austen Thomas, Washington Department of Fish and Wildlife
Ava Fuller, Muckleshoot Tribe
Bruce Stewart, Northwest Indian Fisheries Commission
Casey Clark, University of Washington
Casey Rice, NOAA Northwest Fisheries Science Center
Catherine Morello, R2 Consulting
Clayton David and crew, Pacific Northwest Salmon Center
Clayton Kinsel, Washington Department of Fish and Wildlife
Correigh Greene, NOAA Northwest Fisheries Science Center
Curtis Nelson, Muckleshoot Tribe
Dave Beauchamp, University of Washington
Eric Jeanes, R2 Consulting
Eric Warner, Muckleshoot Tribe
Frederick A. Goetz, Army Corps of Engineers
Gary Marston, Washington Department of Fish and Wildlife
Gina Ylitalo, NOAA Northwest Fisheries Science Center
Jed Moore, Nisqually Tribe
Jesse Nitz, Muckleshoot Tribe
Jason Hall, NOAA Northwest Fisheries Science Center

Josh Chamberlin, NOAA Northwest Fisheries Science Center
Karen Peabody, Washington Department of Fish and Wildlife
Kelly Finley, Tulalip Tribes
Ken Drewes, Muckleshoot Tribe
Kevin Snekvik, Washington State University
Lance Campbell , Washington Department of Fish and Wildlife
Laurie Ann Niewolny , Washington Department of Fish and Wildlife
Madi Gamble, University of Washington
Matt Pouley, Tulalip Tribes
Matthew Klungle, Washington Department of Fish and Wildlife
Mike Crewson, Tulalip Tribes
Mike Mahovlich, Muckleshoot Tribe
Monique M. Lance, Washington Department of Fish and Wildlife
Pete Topping, Washington Department of Fish and Wildlife
Rich Henderson and crew, Skagit River System Cooperative
Sayre S. Hodgson, Nisqually Indian Tribe
Sean Hildebrandt, Muckleshoot Tribe
Sean Sol, NOAA Northwest Fisheries Science Center
Steve Damm, US Fish and Wildlife Service
Thomas P. Quinn, University of Washington
Todd Zackey, Tulalip Tribes

<u>Project Management and Facilitation</u>
Iris Kemp, Long Live the Kings
Michael Schmidt, Long Live the Kings**

* Also members of the US Salish Sea Technical Team, the scientific body of the overarching Salish Sea Marine Survival Project.

**For more information, contact mschmidt@lltk.org

TABLE OF CONTENTS

RESEARCH FINDINGS SUMMARY

Executive Summary

In 2013, the Washington Department of Fish and Wildlife and the Puget Sound Partnership initiated an effort to determine why juvenile steelhead are dying in Puget Sound.[1] This collaborative effort involves state and federal agencies, Puget Sound Treaty Tribes, and academia. It is coordinated by the nonprofit Long Live the Kings and is a component of the Salish Sea Marine Survival Project. The initial phase was funded by a 2013-15 biennium Washington State appropriation of $788,000 via the Puget Sound Partnership 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 Puget Sound Steelhead Marine Survival Workgroup determined that the causes of mortality are most likely derived in the lower-river or marine environments and predation and disease are likely the most significant factors affecting survival. However, how these factors interact, the degree to which these factors affect survival among Puget Sound steelhead populations, and the 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 further investigated.

The next phase of research includes determining the extent of mortality occurring from each source, how the sources of mortality interact, and the specific ecosystem dynamics that lead to this mortality. From here, specific recommendations for management actions will be developed. See the "Puget Sound Steelhead Marine Survival: 2015-2017 Research Work Plan"[2] for more information.

The complete list of primary findings are below, embedded in the research question framework of the Workgroup. The studies and their findings are summarized in this report.

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 compare to other regions? How do the abundance trends, marine survival trends, and early marine mortality rates and locations of mortality vary among populations within Puget Sound?

- Puget Sound steelhead population abundance and marine survival has declined and remain lower than other nearby regions.
- Puget Sound steelhead early marine survival rates are low, with the highest instantaneous mortality rates in South and Central Puget Sound, and the north end of Hood Canal through Admiralty Inlet.
- Typically, the farther steelhead must swim through Puget Sound, the greater the mortality (death by distance traveled).

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

[2] Puget Sound Steelhead Marine Survival Workgroup. December 2015. Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2015-2017 Research Work Plan. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com

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

- A large number of juvenile steelhead are dying quickly in the Puget Sound marine environment, suggesting predation is the source of proximate mortality.
- The list of most likely potential bird and marine mammal predators of outmigrating juvenile steelhead includes harbor seals, harbor porpoises, double-crested cormorants, Caspian terns, and Brandt's cormorants.
- Indirect evidence suggests harbor seals are a source of proximate mortality in South and Central Puget Sound.

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

- The ultimate source of mortality in Central and South Puget Sound is likely marine derived and not associated with freshwater habitat or hatchery influence. However, causes derived in the lower river or fish condition effects consistent among steelhead populations cannot be ruled out.
- *The parasite, Nanophyetus salmincola,* may kill outmigrating steelhead or make them more vulnerable to predation, contributing to lower early marine survival rates of steelhead populations in Central and South Puget Sound. New infections of *N. salmincola* occurring in the lower river are of primary concern.
- PCB's and PBDE's, classes of man-made contaminants, accumulate in some populations of Puget Sound steelhead during freshwater residence, and, due to lipid loss, reach levels during smolt outmigration that may affect their health. PBDE's levels in steelhead leaving the Nisqually River are of primary concern.
- Smolts in some populations with particular genetic fingerprints may be compromised by their morphology (fin development) or immunological responses, making them sick or more vulnerable to predation. However, the power of these findings is currently limited. (Nisqually, Green and Skokomish steelhead were studied.)
- Juvenile steelhead migrating in April and late May survive at higher rates than steelhead migrating in early-mid May. While not yet investigated, this may be associated with factors like changes in predator-prey dynamics or *N. salmincola* shedding events/disease outbreaks.
- Steelhead foraging-predation rate relationships weren't investigated, but starvation isn't likely.
- Whole body lipid content was 1.5% or less in the wild Puget Sound steelhead populations that were assessed. Low lipid levels are not inconsistent with a decline in whole body lipid content toward depletion during the smolt outmigrant life-stage. However, levels below 1% were observed in some Puget Sound steelhead, and this may be cause for concern as 1% has been documented as a threshold for the onset of high over-winter mortality in rainbow

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

trout. Additional analyses are planned to assess whether the prevalence and intensity of *N. salmincola* affects lipid levels in steelhead smolts.

- Juvenile steelhead size at outmigration and steelhead outmigrant abundance are not correlated with survival among years. Size at outmigration is also not correlated with survival within years.
- An increase in the abundance of harbor seals correlates with the decline in steelhead survival. Abundance data are lacking for a correlative assessment of the other potential predators; however, qualitative information suggests there may be less of an association with the decline in steelhead survival.
- Changes in herring abundance, water clarity, and abundance of hatchery salmon over the period of the decline steelhead marine survival may be affecting predator-prey dynamics.

Please visit www.marinesurvivalproject.com for more information.

Introduction

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 a key factor in that decline and a significant barrier to recovery.

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

In 2013, the Washington Department of Fish and Wildlife and Puget Sound Partnership 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)[4] 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[5]. The initial research phase was funded by a 2013-15 biennium appropriation of $788,000 via the Puget Sound Partnership and $800,000 of direct match in equipment, services, and staff time from collaborators.

This report summarizes the findings from the 2013-2015 research phase. Extended abstracts of each study are included. *Several studies described in this document are subject to further revisions prior to publication in peer-reviewed journals.* Published studies and technical reports are available on the

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

[5] 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.

resources page of www.marinesurvivalproject.com. As manuscripts and reports are completed, they will continue to be made available via the Salish Sea Marine Survival project web site.

Research Framework

To develop the 2013-2015 research, the Workgroup reviewed, discussed, and categorized the existing evidence and developed their research assumptions based upon the following framework.

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[6] 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 assumptions are summarized in the diagram below (**Figure 1**). Evidence supporting the assumptions is detailed in the Research Work Plan: Marine Survival of Puget Sound Steelhead (2014)[7]. In general:

(Q1) Through initial work, the Workgroup found disparate trends and lower smolt-to-adult (marine) survival for Puget Sound steelhead populations compared to those from Washington Coast or the Columbia River. They also found, from acoustic telemetry studies in 2006-2009, that high and rapid juvenile steelhead mortality occurred in Puget Sound. Finally, initial investigations of abundance, smolt-to-adult survival, and early marine mortality data suggested higher mortality for steelhead that travel farther through Puget Sound (those from south Puget Sound or south Hood Canal experience the highest mortality rates).

(Q2) Based on the existing evidence from acoustic telemetry studies showing rapid mortality in Puget Sound, the Workgroup concluded that predation is the most likely proximate source of this mortality. They did not completely rule out other factors that could lead directly to mortality, and concluded those could be assessed peripherally via the studies of ultimate causes of mortality (Q3).

(Q3) While changes in predator abundance could be fundamentally driving steelhead survival in Puget Sound, the Workgroup concluded that a comprehensive assessment of root or ultimate causes was warranted. The Workgroup generally agreed that no one factor is likely working in isolation, and it is the combination of specific factors leading to high mortality rates that must

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

[7] 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

be determined. These ultimate causes/factors were separated into two groups: 1) those that directly affect predator-prey interactions, and 2) those that compromise steelhead condition/health or alter their outmigrant behavior (which could then expose steelhead to higher predation rates or to direct mortality). Factors were further categorized by whether they were freshwater or marine-derived. Based upon existing evidence, the Workgroup then ranked causes for poor fish health/altered behavior. Disease was the factor ranked most likely to be compromising steelhead health or altering their outmigrant behavior. Toxic contaminants and a genetic basis for predisposition to mortality were ranked 2nd and 3rd.

Since disease is a broad category, the Workgroup convened fish health experts from the Puget Sound region to prioritize the pathogens and parasites of greatest concern. *Nanophyetus salmincola* was deemed the strongest candidate because of its high prevalence and intensity among other salmonids in the watersheds with the lowest steelhead smolt-to-adult survival rates and highest early marine mortality rates (south Puget Sound and south Hood Canal). *N. salmincola* infections could result in rapid mortality shortly after seawater entry. In particular, the literature shows new infections of *N. salmincola* decrease swimming performance, which could lead to increased predation rates.

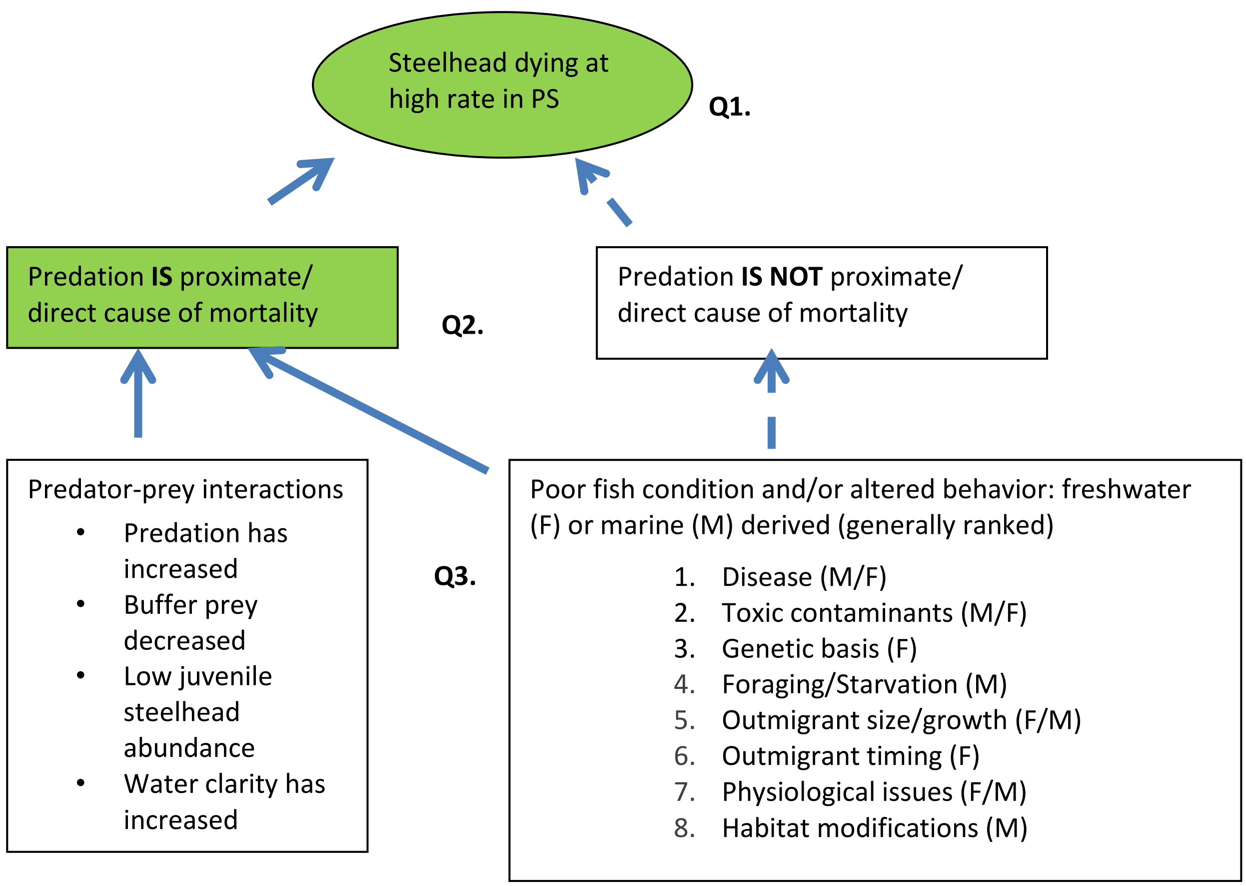

Figure 1. Puget Sound steelhead marine survival evaluation: The green color indicates where the group generally agrees with the evidence. The factors that may be affecting fish condition or behavior are also ranked based upon existing evidence (from Salish Sea Marine Survival Project - Research Work Plan: Marine Survival of Puget Sound Steelhead (2014)).

2013-2015 Research Components

Ten studies were implemented during the 2013-2015 Washington State biennium to improve the Workgroup's answers to the three questions that constitute the framework of the 2013-2015 research work plan. The studies are categorized in response to the three questions; however, several studies addressed more than one question, as illustrated in the findings section below. Extended abstracts of the specific studies and their findings are provided in the appendices section of this report:

(Q1) The Workgroup concluded additional work should be done to assess the spatial patterns and temporal trends of steelhead mortality using existing abundance, smolt-to-adult (marine) survival, and telemetry/early marine mortality data to: a) establish datasets for assessing correlations with steelhead fish characteristics and environmental characteristics; and b) help further isolate where, when, and at what rate mortality is occurring. Two studies were preformed:

Study 1: Multi-population analysis of Puget Sound steelhead survival and migration behavior

Study 2: Western Washington State steelhead trout (*Oncorhynchus mykiss*) spawner abundance and marine survival trends

(Q2) A draft list of likely predators was created during this initial round of research, and the Workgroup concluded the list should be formalized through further study of existing data. As harbor seals were considered a strong candidate predator early in the research development process (but not the only predator), the Workgroup concluded that an assessment of harbor seal-steelhead interactions was warranted. Two studies were performed:

Study 6: Identifying Potential Juvenile Steelhead Predators in the Marine Waters of the Salish Sea

Study 7: Predator-prey interactions between harbor seals and migrating steelhead smolts revealed by acoustic telemetry

(Q3) The Workgroup concluded that the suite of potential factors causing weak steelhead survival could be reduced via a high-level study that helps determine whether the underlying causes of mortality are freshwater or marine derived. They also concluded that existing data could be used to perform a correlative analysis comparing smolt-to-adult survival patterns and trends to steelhead fish characteristics and environmental characteristics. To test the highest ranked factors that may affect fish condition, a broad assessment of fish health was planned, focusing primarily on *N. salmincola a*nd toxic contaminants and building from the rivers (freshwater) through to the offshore (marine). Finally, the Workgroup determined that a genome-wide association study (GWAS) could be performed to determine whether there are genomic differences between outmigrating steelhead smolts that survived to the open ocean (exiting Strait of Juan de Fuca) versus those smolts that died somewhere within Puget Sound. This study would utilize DNA samples collected from acoustically-tagged smolts in 2006-2009 and 2014. Ultimately, six studies were performed.

Study 3: Fish characteristics and environmental variables related to marine survival of

Western Washington State steelhead trout (*Oncorhynchus mykiss*)

Study 4: Geographic location outweighs effects of freshwater rearing and hatchery influence on early marine survival of Puget Sound steelhead

Study 5: Steelhead smolt releases from Skagit River used to estimate detection efficiency of Strait of Juan de Fuca acoustic telemetry line

Study 8: Prevalence and load of *Nanophyetus salmincola* infection in outmigrating steelhead trout from five Puget Sound rivers

Study 9: Toxic contaminant exposure in juvenile Puget Sound steelhead

Study 10: Genome-wide association study of acoustically tagged steelhead smolts in the Salish Sea: measuring differences between survivors and non-survivors

2013-2015 Findings

The findings of the ten studies are summarized below and organized in accordance with the research framework. For additional details, please reference the extended abstracts for each study in the appendices. Manuscripts or technical reports are being completed for each study and will be made available at www.marinesurvivalproject.com/resources. Note also that, for the most part, the evidence that established the Workgroup's initial position on the factors affecting survival, leading to the 2013-2015 research, is not repeated below. See "Salish Sea Marine Survival Project - Research Work Plan: Marine Survival of Puget Sound Steelhead" on the resources page of marinesurvivalproject.com for this complementary evidence.

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 in Puget Sound compare to other regions? How do abundance and marine survival trends, and early marine mortality rates and locations of mortality vary among populations within Puget Sound?

Puget Sound steelhead population abundance and marine survival has declined and remains lower than other nearby regions - Spatially-explicit trends in steelhead abundance and smolt-to-adult survival rates (SARs, a.k.a. marine survival rates) were developed for hatchery and wild populations from Puget Sound, the Washington coast and the Columbia River, dating back to the 1970s (Study 2). MARSS (Multivariate Auto-Regressive State-Space) models were used to assess whether population dynamics vary among the regions. The results confirmed that Puget Sound populations have distinct trends compared to populations from other nearby regions. Furthermore, Puget Sound steelhead marine survival rates have generally been lower and have not rebounded as much as populations from other regions.

Puget Sound steelhead early marine survival rates are low, with the highest instantaneous mortality rates in South and Central Puget Sound, and the north end of Hood Canal through Admiralty Inlet - Data collected from several juvenile steelhead telemetry studies that occurred across eight rivers in Hood Canal and Puget Sound were re-examined (Study 1). The results indicate early marine survival rates (from river mouth through the Strait of Juan de Fuca) ranged from 0.8% to 39.3%, and averaged 16.0% for wild smolts and 11.4% for hatchery smolts over the four years of the study (2006-2009). The 2006-2009 data also indicate that steelhead smolts suffered greater instantaneous mortality rates (based on a combination of mortality rates and travel time through specific migration segments) in the central region of Puget Sound and from the north end of Hood Canal through Admiralty Inlet than in other monitored migration segments. Furthermore, results from study 4 indicate early marine survival rates of 5.9 ± 4.2% to 17.4 ± 7.1% for steelhead released from the Nisqually and Green rivers, respectively. Results from study 4 corroborate the 2006-2009 data: instantaneous mortality rates were greatest in South Puget Sound, and mortality was highest from river mouth through Admiralty Inlet (within Puget Sound proper).

Typically, the farther Puget Sound steelhead must swim through Puget Sound, the greater the mortality (death by distance traveled) - Puget Sound steelhead abundance trends support the hypothesis that steelhead survival is worse for populations that have to travel farther through Puget Sound (those entering South and Central Puget Sound compared to populations entering the more northern Whidbey and Rosario basins). This pattern can also be seen in the acoustic telemetry studies (Study 1 and 4), where Nisqually and Skokomish steelhead--in Puget Sound and Hood Canal, respectively—experience the lowest early marine survival rates. In study 4, the location of the river mouth within Puget Sound had the greatest bearing on survival of steelhead smolts through Puget Sound; smolts with shorter migration distances survived at a higher rate than those with longer distances to migrate. Finally, in study 5, an assessment of the early marine survival rates of Skagit steelhead showed that fish taking the shorter migration route to the Pacific Ocean, through Deception Pass and the Strait of Juan de Fuca (119km), survived at 1.7 times the rate of those that took the longer, southern migration route through Saratoga Passage, around Whidbey Island and then northwest through Admiralty Inlet and the Strait of Juan de Fuca (207km).

In study 5, V7 tag detection efficiency of the Strait of Juan de Fuca telemetry line was also tested, comparing the standard tags used in most of the steelhead studies (V7 tags) to larger, more powerful tags that have 100% detection efficiency (V9 tags). The results showed that 66.7% of the V7 tags were detected by the Strait of Juan de Fuca line. The agreement of this empirical estimate with modeled line efficiency rates of 68.5% (Melnychuk 2009)[8] increased confidence in survival estimates based on V7 tag studies.

[8] Melnychuk MC (2009) Mortality of migrating Pacific salmon smolts in Southern British Columbia. PhD thesis. University of British Columbia, Vancouver.

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

A large number of juvenile steelhead are dying quickly in the Puget Sound marine environment, suggesting predation is the source of proximate mortality – Studies 1 and 4 showed juvenile steelhead travel rapidly through the estuary and marine environments of Puget Sound. In Study 1, average migration times from river mouth through the Strait of Juan de Fuca ranged from only 6.2 days (Green River population) to 18.1 days (Skokomish River population). In study 4, travel times from river mouth through the Strait of Juan de Fuca were – A) Nisqually releases: 257 km in 9.80 ± 1.19 days. B) Green releases: 187 km in 8.80 ± 0.44 days). In study 5, the average travel time for Skagit steelhead smolts from Skagit Bay through the Strait of Juan de Fuca was 5 days. Rapid outmigration rates, coupled with high freshwater survival and low Puget Sound marine survival rates, suggest a source of mortality that acts quickly on a large number of smolts in the Puget Sound marine environment. Predation fits this pattern well. Substantial indirect evidence from studies 4 and 7, described in further detail below, supports predation as the proximate source of mortality.

Other sources of proximate, instantaneous mortality could include contaminants, harmful algae blooms, or disease. However, based upon the results of the study 9, it is unlikely that contaminants cause direct mortality. Contaminant levels in outmigrating Puget Sound steelhead are lower than mortality thresholds (study 9). Study 8 found that the prevalence and intensity of *Nanophyetus salmincola* infections are high for juvenile steelhead outmigrating from the Nisqually and Green rivers. A laboratory study did not provide any indication that heavy *N. salmincola* loads would result in instantaneous mortality during seawater transition. However, the logistics of the laboratory study resulted in a lag of three weeks between *N. salmincola* exposure and seawater challenge, with the experimental seawater transition occurring after the most pathogenic stages of infection. Therefore, the Workgroup will be retesting whether new infections of *N. salmincola* could result in instantaneous mortality during the 2015-2017 study period. Finally, as stated in the Puget Sound steelhead marine survival research work plan for 2014[9], acoustic telemetry and SAR data indicate that mortality is not highly variable among years and occurs throughout Puget Sound, suggesting that mortality is not caused by factors with high spatial and temporal variability in the environment such as harmful algae blooms. Additional retrospective work was recommended to support or refute this hypothesis. However, data are limited.

The list of most likely potential bird and marine mammal predators of outmigrating juvenile steelhead includes harbor seals, harbor porpoises, double-crested cormorants, Caspian terns, and Brandt's cormorants - Potential marine mammal and bird predators of out-migrating juvenile steelhead were identified in study 6 based on predator distribution, abundance, and diet information. Based upon the literature review, harbor seals, double-crested cormorants, Caspian terns, and Brandt's cormorants are the most likely potential predators. The results are described in detail in the affiliated technical report, available on the resources page of www.marinesurvivalproject.com. [10] These fish-eating species have demonstrated relatively stable or increasing population trends in recent years (over the same period as

[9] 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

[10] Pearson, S.F., S.J. Jeffries, M.M. Lance and A.C. Thomas. 2015. Identifying potential juvenile steelhead predators in the marine waters of the Salish Sea. Washington Department of Fish and Wildlife, Wildlife Science Division, Olympia.

the decline in Puget Sound steelhead marine survival) and their diet includes juvenile salmon and steelhead.

Double-breasted cormorants may be of lower concern because a large portion of the population migrates to the Columbia River in late April for mating season, before the peak of the juvenile steelhead outmigration period. Although, it is possible that immature birds (one and two- year olds) may linger in the Sound longer than adults since they do not fully populate the Columbia River breeding colonies until mid-June. [11] Anecdotally, the presence of Caspian tern nesting has been variable in Puget Sound in recent years, and the May nesting period coincides with steelhead outmigration. [12] The abundance of harbor seals has increased substantially in Puget Sound and the greater Salish Sea over the period of steelhead decline. [13] Study 3 illustrates the inverse relationship between seal abundance and Puget Sound steelhead marine survival. The relative abundance and distribution of harbor seals during the April-June steelhead outmigration period has not been established; however, it is a priority.

Although juvenile salmon and steelhead have not been detected in stomach contents in Puget Sound, harbor porpoise sightings have increased dramatically during the period of steelhead decline and, because porpoises find their prey using echolocation, they have a unique ability to exploit a resource like juvenile steelhead that tend to move individually or in small groups. That said, the substantial increase in harbor porpoise sightings began in the late 1990's, after the period during which Puget Sound steelhead marine survival declined rapidly (however, the harbor porpoise data over the period of steelhead decline are very coarse). [14] Recent dive data from sea lions in South Puget Sound during the steelhead outmigration period suggest that sea lions are mainly foraging deep in the water column, at lower depths than where juvenile steelhead outmigrate.[15]

Indirect evidence suggests harbor seals are a source of proximate mortality in South and Central Puget Sound – Study 7 investigated predator-prey interactions between harbor seals and juvenile steelhead migrating through Puget Sound using acoustic telemetry (246 tagged steelhead and 11 seals with mounted receivers). The study resulted in the first data suggesting harbor seals consume juvenile steelhead in Puget Sound. The study showed that harbor seals and migrating steelhead have substantial spatial and temporal overlap, and provided indirect evidence of harbor seal predation events via tag detection patterns (repeated detections over 3-4 day period consistent with gut passage time for harbor seal, and tags detected as stationary (deposited) near harbor seal haulout sites). Detection data did not suggest any tagged smolts were ingested by seals with mounted receivers, and we cannot rule out that tags may have been deposited near haulout sites by other predators. The study also did not result in an estimate of the overall predation rate by seals on migrating juvenile steelhead. The potential for a dinner bell effect (pinging tags attracting harbor seals and biasing results) was also tested in study 7. There was no evidence for effects of tag noise on survival of steelhead smolts; however, the sample size was small.

[11] Ibid.
[12] Ibid.
[13] Ibid.
[14] Ibid.
[15] pers. comm. S. Jeffries, Washington Department of Fish and Wildlife, June 2015.

Study 4, which also assessed acoustically-tagged steelhead, provided additional indirect evidence of predation by harbor seals. Although not described in the extended abstract appended to this document, some of the acoustic tags were detected moving back and forth with the tides, through the Nisqually estuary and nearby marine environment, and not detected again by the receiver arrays in Puget Sound. This pattern is consistent with harbor seal behavior in estuaries, suggesting the tagged steelhead were consumed by a harbor seal and the harbor seal was detected by the receivers in the estuary and marine environment.

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

As stated above, ultimate causes/factors were investigated in two groups: 1) those that directly affect predator-prey interactions, and 2) those factors that compromise steelhead condition/health or alter their outmigrant behavior (which could then expose steelhead to higher predation rates or to direct mortality). Factors were further isolated by whether they were freshwater- or marine-derived.

The ultimate source of mortality in Central and South Puget Sound is likely marine-derived and not associated with freshwater habitat or hatchery influence. However, causes derived in the lower river or fish condition effects consistent among steelhead populations, cannot be ruled out – Study 4 took advantage of contrasting conditions in geographically proximate river systems to test for the effects of freshwater rearing conditions and hatchery introgression on survival rates of steelhead migrating from river mouth to the Pacific Ocean (Green River = degraded habitat and current hatchery influence, Nisqually River = high quality habitat and no current hatchery steelhead influence). Steelhead smolts were cross-planted from one river to another and compared to plants into natal rivers to determine whether low early marine survival rates could be due to population-specific effects like freshwater rearing conditions or hatchery introgression, or if direct effects within the marine environment were more likely the cause. Similar survival probabilities among smolts released in the Green and Nisqually rivers, despite clear differences in freshwater habitat and hatchery influence, render these factors unlikely to substantially influence early marine survival of these populations. However, because the fish were released at river kilometer 19 in both systems, factors affecting steelhead in the lower river, if immediate and at a high rate (e.g., disease and contaminants), could still explain similarities in mortality of the two reciprocally transplanted populations. Furthermore, although less likely, underlying drivers of fish condition could be the root cause of, or contributing to, the mortality. However, these underlying drivers would have to be consistent among populations to pair with the results of study 4.

Fish condition, the factors affecting condition or altering behavior, and their potential role in juvenile steelhead mortality

The parasite*, Nanophyetus salmincola,* may kill outmigrating steelhead or make them more vulnerable to predation, contributing to lower early marine survival rates of steelhead populations in Central and South Puget Sound. New infections of N. salmincola occurring in the lower river are of primary concern. – Study 8 compared the prevalence and intensity of *N. salmincola* and other diseases in five steelhead populations throughout Puget Sound (Skagit, Snohomish, Green, Nisqually) and Hood Canal (Tahuya). The prevalence and parasite loads of *N. salmincola* were significantly higher in outmigrating steelhead smolts from central and south Puget Sound watersheds (Green and Nisqually) than in those from north Puget Sound (Skagit and Snohomish), where infections were rarely detected. *N. salmincola* was also not found in any smolts from the Tahuya watershed. The Green and Nisqually Rivers had high prevalence and parasite loads (above reported thresholds for negative health effects), and a substantial portion of fish from these rivers with *N. salmincola* also exhibited gill (Green 28%, Nisqually 42%) and heart (Green 45%, Nisqually 69%) inflammation not found in the other three rivers. A downstream progression of *N. salmincola* prevalence and intensity in steelhead, and high prevalence and intensity of *N. salmincola* in steelhead captured in the estuaries, suggests that new infections of *N. salmincola* may be occurring as juvenile steelhead move downstream and out into Puget Sound during their migration. Furthermore, substantial differences in *N. salmincola* prevalence between Green (13.3%) and Nisqually (98-100%) steelhead captured at the in-river trap sites combined with the results of study 4 (similar early marine survival rates of steelhead captured at these trap sites and reciprocally transplanted) further suggest that host survival may be influenced by novel *N. salmincola* exposures that occur in the lower portions of the watersheds. The presence of new infections occurring in the lower river/estuaries of the Green and Nisqually, and heart and gill inflammation found in the steelhead, may be killing the steelhead outright in Puget Sound or, more likely, compromising their ability to swim as they enter and migrate through Puget Sound and increasing their susceptibility to predation.

Furthermore, study 8 suggests *N. salmincola* infections may help explain the difference in early marine survival between hatchery and wild steelhead from the same watershed. While hatchery and wild steelhead both had heavy parasite burdens in the Green River, lower early marine survival rates (and SARs) among hatchery cohorts may be associated with their prolonged residence in lower river / estuarine habitats, where additional exposures are likely to occur.

Finally, histology was performed to investigate the prevalence of other diseases in study 8. While other diseases were found, none other than *N. salmincola* were considered to be consistent with Puget Sound early marine mortality patterns.

It should be noted here that *N. salmincola* does not explain the early marine mortality rates experienced by steelhead in Northern Puget Sound, or those in the Strait of Georgia. Furthermore, based upon the results of study 2, there is a stronger association in patterns of smolt-to-adult survival between North Puget Sound and Central & South Puget Sound populations than between North Puget Sound and coastal or Columbia River populations. Therefore, the degree to which *N. salmincola* contributes to early marine mortality must be examined.

PCB's and PBDE's, classes of man-made contaminants, accumulate in some populations of Puget Sound steelhead during freshwater residence, and, due to lipid loss, reach levels during smolt outmigration that may affect their health. PBDE's levels in steelhead leaving the Nisqually River are of primary concern – Study 9 investigated contaminant loads in the three of the four Puget Sound steelhead populations referenced in Study 8: Skagit, Green, Nisqually. The Snohomish, Hood Canal, and Tahuya populations were not included. Results show that man-made, persistent organic pollutants are generally below concentrations associated with adverse effects. PCB or PBDE[16] levels did exceed

[16] commonly referred to as flame retardants

potentially harmful levels up to 17-25% and 50%, respectively, of samples from steelhead recovered in the North/Whidbey Basin, Central and South Puget Sound offshore marine habitats. However, PCB concentrations were low within the Skagit, Green and Nisqually rivers and their associated estuaries. The increase in harmful PCB concentrations offshore is primarily due to lower fish lipid content as migration proceeded. In contrast, 33% of the steelhead collected in the in-river trap and the estuary of the Nisqually River had PBDE levels that could increase disease susceptibility or alter thyroid production. However, to be consistent with the results of study 4 that suggest freshwater habitat isn't affecting early marine survival, Nisqually steelhead may need to be impacted by these PBDEs rapidly via exposure below river kilometer 19, the release site for study 4.

Smolts in some populations with particular genetic fingerprints may be compromised by their morphology (fin development) or immunological responses, making them sick or more vulnerable to predation. However, the power of these findings is currently limited. (Nisqually, Green and Skokomish steelhead were studied.) – A genome-wide association study (GWAS) was performed to investigate potential differences in genotypes associated with steelhead that survived to the Strait of Juan de Fuca receiver array vs. those that we presume died along the way (Study 10). This was performed by analyzing DNA samples taken prior to release of acoustic-tagged steelhead in past years. The final dataset included samples from the Skokomish, Green and Nisqually rivers after removing sample sets that may confound the results. Although the study lacked power to provide a definitive association between smolt genotypes and smolt fate (survival/mortality in Puget Sound), study 10 suggests there may be a difference between survivors and mortalities involving morphological features that may affect swimming performance (axial and fin development) and in the capacity for a fish to respond to pathogens or parasites. Among other things that resulted in the lack of power, the lack of independence between year and source and between source and release warrant additional investigation since a small subset of the fish analyzed could be driving the results. Finally, it should be noted that the Nisqually, Green, and Skokomish rivers all have *N. salmincola*.

Juvenile steelhead migrating in April and late May survive at higher rates than steelhead migrating in early-mid May. While not yet investigated, this may be associated with factors such as changes in predator-prey dynamics or *N. salmincola* shedding events/disease outbreaks - Based upon the results of study 1, outmigration timing was an important factor driving wild steelhead smolt survival. Steelhead smolts migrating in early April and late May had a higher probability of survival than those released in early and mid-May. Furthermore, study 4 showed that steelhead from the Nisqually population migrating earlier (late April) survived better than those migrating later (though no difference in survival by release date was observed for smolts of Green River origin). This could be associated with several different factors. Predators may not be keying in on outmigrants until the peak of the steelhead outmigration period or when both hatchery coho and wild steelhead are available. Alternatively, earlier (or later) outmigrants may avoid *N. salmincola* shedding events in the lower river.

A steelhead foraging-predation rate relationship was not investigated, but starvation is not likely –See the description on p. 25 of the Workgroup's initial research work plan regarding steelhead foraging behavior and the unlikelihood of starvation.[17] Telemetry data are not consistent with steelhead foraging behavior (indicated by vertical or back and forth movement at the telemetry receiver arrays), and the rapid outmigration rate and uniform direction of migration is consistent with steelhead outmigration patterns in other regions with higher steelhead survival, such as the Columbia River estuary. However, rapid migration could be induced by a lack of food in a particular area and could lead to increased exposure to predation.[18] Therefore, forage-induced predation cannot be ruled out.

Whole body lipid content was 1.5% or less in wild Puget Sound steelhead populations that were assessed. Low lipid levels are not inconsistent with a decline in whole body lipid content toward depletion during the smolt outmigrant life-stage. However, levels below 1% were observed in some Puget Sound steelhead, and this **may be cause for concern as 1% has been documented as a threshold for the onset of high over-winter mortality in rainbow trout** – Whole body lipid content was analyzed in wild steelhead in study 9 as a metric of fish condition. The results indicate that the pooled samples analyzed had levels at or less than 1% for three rivers (Nisqually, Green, Skagit) assessed. Low lipid levels are a natural function of the spring smolt outmigrant life stage. During the smolt stage, energy is heavily used for growth and migration vs. stored as fat, and there is a decline in whole body lipid content toward depletion.[19,20] However, smolt lipid levels lower than 1% were not documented in the papers reviewed.[21,22,23] Lipid levels below 1% have been associated with the onset of high over-winter mortality in rainbow trout.[24] Low lipid levels can also exacerbate disease and contaminant loads, and can be a sign of poor overall fish condition. That said, hatchery steelhead, which are fed until release and likely have higher lipid levels, do not have higher early marine survival than wild steelhead (see study 1). Additional analyses are planned to assess whether the prevalence and intensity of *N. salmincola* affects lipid levels in steelhead smolts.

Juvenile steelhead size at outmigration and steelhead outmigrant abundance are not correlated with survival among years. Size at outmigration is also not correlated with survival within years - Study 3 used available data describing fish characteristics and environmental variables to investigate correlations with steelhead SARs/marine survival trends. Smolt weight, recorded for hatchery releases,

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[17] 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.

[18] pers. comm. C. Walters December 2014. December 2014 Salish Sea Marine Survival Project, US-Canada Retreat.

[19] Sheridan, M.A., V. Allena ND, T.H. Kerstetter. 1983. Seasonal variations in the lipid composition of the steelhead trout, *Salmo gairdneri* Richardson, associated with the parr- smolt transformation. Journal of Fish Biology: 23, 125-134.

[20] Stefansson, B.T. Bjornsson, K. Sundell, G. Nyhammer, S.D. McCormick. 2003. Physiological characteristics of wild Atlantic salmon post-smolts during estuarine and coastal migration. Journal of Fish Biology. 63:942-955.

[21] Sheridan et. al. AND Stefansson et. al. (see above)

[22] Fessler, J.A. 1969. Some morphological and biochemical changes in steelhead trout during the parr-smolt transformation. Thesis. Oregon State University.

[23] McMillan, J.R., G.H. Reeves, C.E. Jordan. 2011. Individual condition and stream temperature influence on early maturation of rainbow and steelhead trout, Oncorhynchus mykiss. Environmental Biology of Fish. DOI 10.1007/s10641-011-9921-0

[24] Biro, P.A., A.E. Morton, J.R. Post, E.A. Parkinson. 2003. Over-winter lipid depletion and mortality of age-0 rainbow trout (Oncorhynchus mykiss). Canada Journal of Fisheries and Aquatic Sciences. **61**: 1513–1519.

showed no correlation with overall marine survival. This is consistent with early marine survival acoustic telemetry studies 1 and 4, where fork length was not correlated with early marine survival, showing no evidence of size selective mortality that would have derived in freshwater. Study 3 also found no correlation between steelhead outmigrant abundance (smolt count/hatchery release number) and overall marine survival.

Factors affecting predator-prey dynamics in the marine environment

An increase in the abundance of harbor seals correlates with the decline in steelhead. Abundance data are lacking for a correlative assessment of the other potential predators – See the description of predators under Q2, above, for details.

Changes in herring abundance, water clarity, and abundance of hatchery salmon over the period of the decline in Puget Sound steelhead marine survival may be affecting predator-prey dynamics – Other basin-specific relationships between specific environmental indicators and steelhead marine survival were found in study 3. They included a positive correlation between adult herring abundance and steelhead marine survival, and negative or inverse correlations between hatchery coho abundance and steelhead marine survival and marine survival. The correlation with herring abundance could be a predator buffer effect, or possibly herring and steelhead are similarly affected by another factor. Increased water clarity (reduced turbidity) is well documented to potentially lead to increased predator-prey encounter rates.[25]

Next steps

The revised logic model, based upon these new findings, is below. A crosswalk between the revised logic model and the findings is included as an appendix to this document. An additional Washington State appropriation of $800,000 was provided via the Washington Department of Fish and Wildlife to continue this work in the 2015-2017 biennium. The next phase of research will build upon the findings to date. The work will include determining the extent of mortality occurring from each source, how the sources of mortality interact, and the specific ecosystem dynamics that lead to this mortality. From here, specific recommendations for management actions will be developed. The work will be described in the forthcoming research work plan for the 2015-2017 biennium.

[25] Described in: 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.

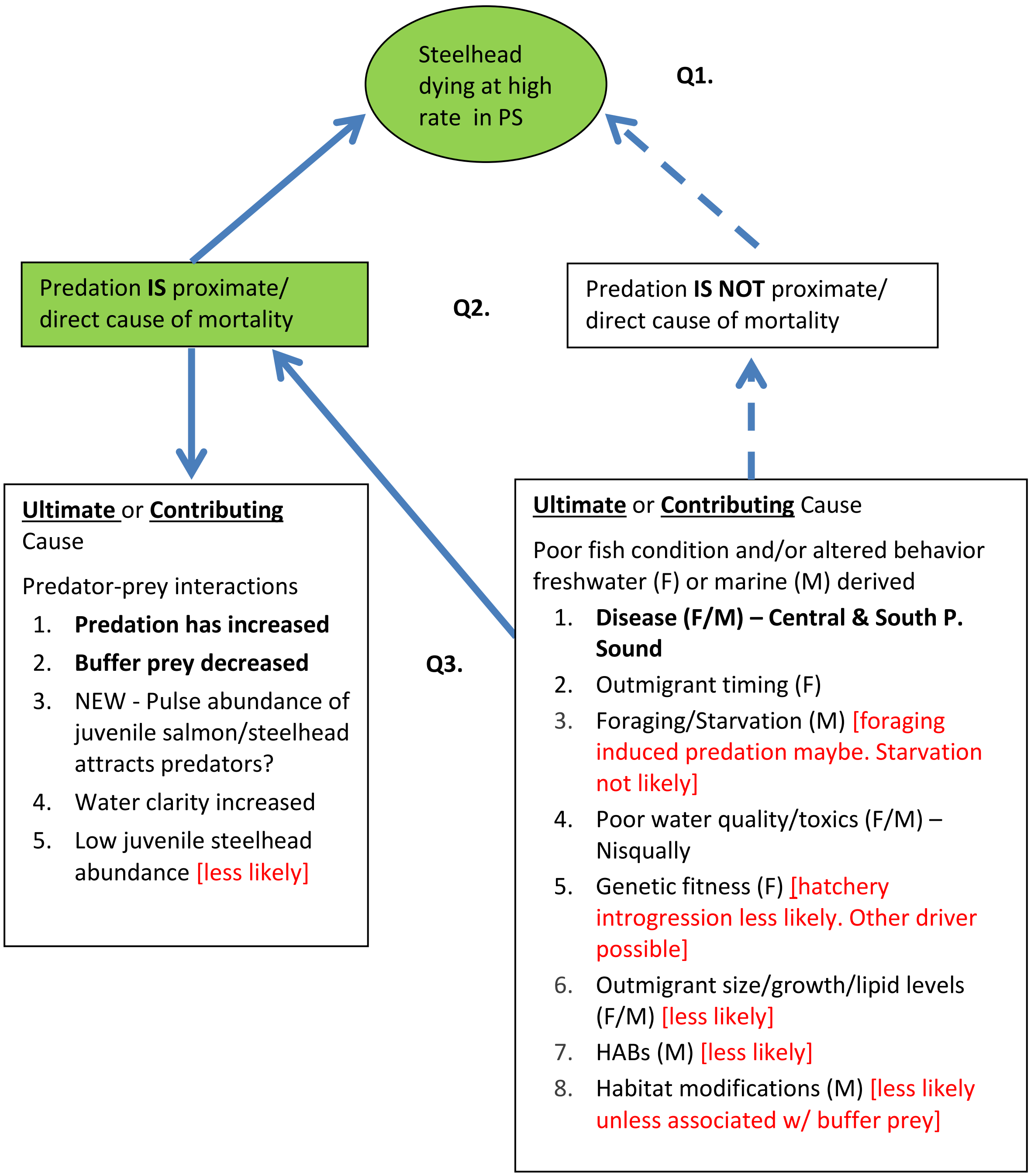


Figure 2. Updated Puget Sound steelhead marine survival evaluation. The factors are ranked based upon existing evidence.

APPENDIX A: EXTENDED ABSTRACTS

Study 1: Multi-population analysis of Puget Sound steelhead survival and migration behavior

Megan E. Moore[1], Barry A. Berejikian[1], Frederick A. Goetz[2], Andrew G. Berger[3], Sayre S. Hodgson[4], Edward J. Connor[5] and Thomas P. Quinn[6]

[1]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration Fisheries, P.O. Box 130, Manchester, 98353, USA

[2]U.S. Army Corps of Engineers, 4735 East Marginal Way South, Seattle WA 98134

[3]Puyallup Tribe Fisheries Department, Puyallup Tribe of Indians, 3009 East Portland Avenue, Tacoma WA 98404

[4]Department of Natural Resources, Nisqually Indian Tribe, 4820 She-Nah-Hum Drive, Olympia WA 98513

[5]Seattle City Light, City of Seattle, 700 Fifth Ave. Seattle WA 98104, USA

[6]School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle, WA 98195

Telemetry studies of steelhead (*Oncorhynchus mykiss*) smolts in Puget Sound, Washington, U.S.A. have indicated that approximately 80% of fish entering marine waters do not survive to reach the Pacific Ocean. This level of early marine mortality may limit recovery of threatened Puget Sound steelhead. The present study re-examined data from previous steelhead telemetry research in the Hood Canal region of Puget Sound (Dewatto River, Hamma Hamma River, South Fork Skokomish River and Big Beef Creek populations) and incorporated data from additional Puget Sound populations (Skagit River, Green River, Puyallup River, and Nisqually River populations) tagged during the same time period (2006-2009) for a comprehensive analysis of steelhead early marine survival.

Early marine survival probabilities (river mouth to the Strait of Juan de Fuca) ranged from 0.8% (Skokomish hatchery population in 2009) to 39.3% (Big Beef Creek wild population in 2006), and averaged 16.0% for wild smolts and 11.4% for hatchery smolts over the four years of the study (Figure 1). Mark-recapture model comparison indicated that an interaction between rear type (wild or hatchery) and population factors plus a year effect best explained differences in survival among the tagged populations. Hatchery smolt survival was lower than wild smolt survival in some populations but not others. Release date was an important factor driving wild smolt survival; smolts migrating in early April and late May had a higher probability of survival than those released in early and mid-May. Inclusion of fork length in survival models did not provide evidence of substantial size selective mortality within the freshwater environment or in Puget Sound.

Freshwater survival probabilities of all monitored populations were substantially higher than survival probabilities calculated for similar distances in the marine environment. Steelhead smolts suffered greater instantaneous mortality rates in the central region of Puget Sound and from the north end of Hood Canal through Admiralty Inlet than in other monitored migration segments.

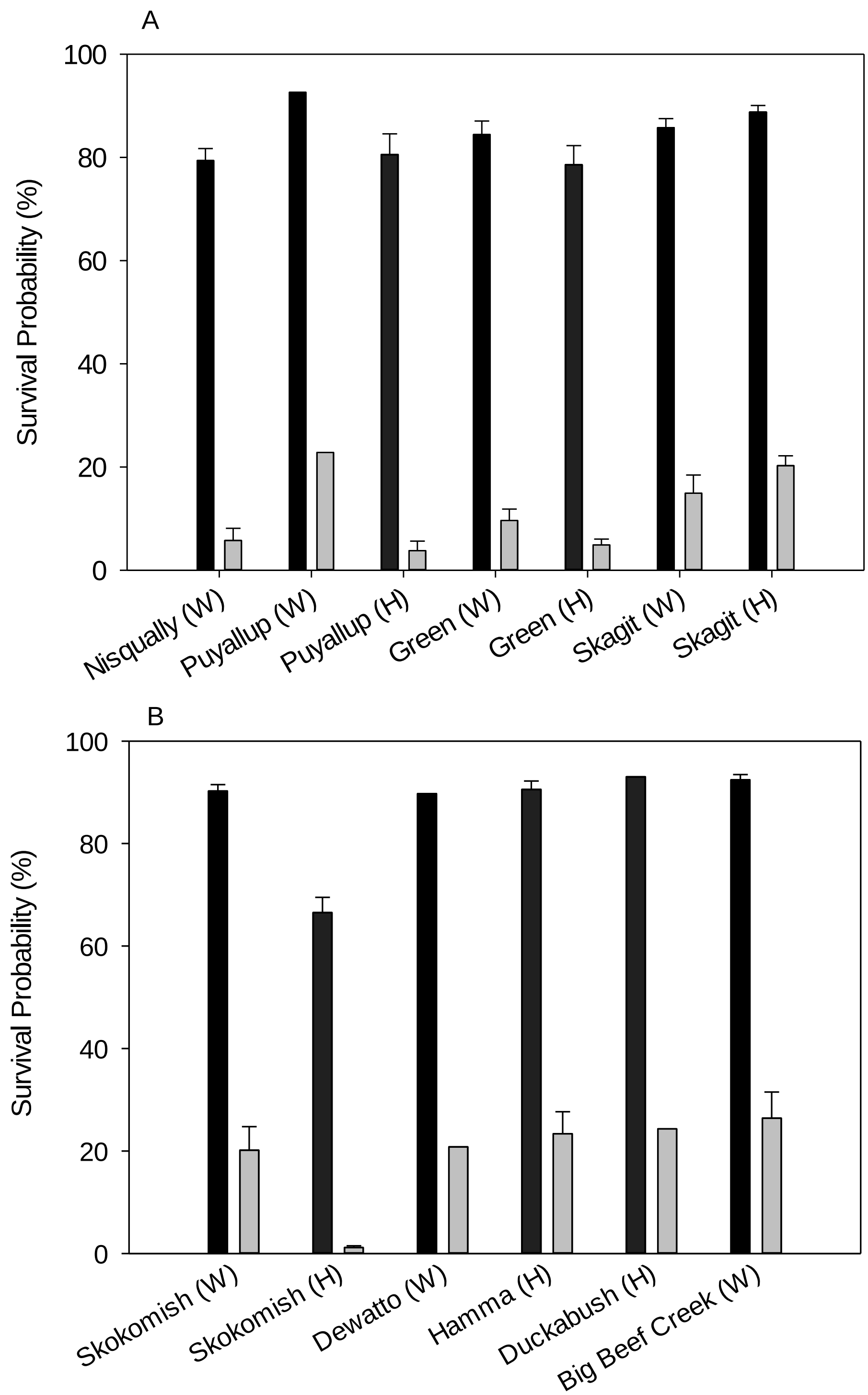
Smolts from all populations spent little time in estuarine areas and travelled rapidly from river mouth to the Strait of Juan de Fuca. Population averages ranged from only 6.2 days (Green River smolts) to 18.1 days (Skokomish River).

Short residence times, coupled with the high freshwater and low Puget Sound survival probabilities observed in this study, suggest a source of mortality that acts quickly on a large number of smolts in the early marine environment. Predation fits this pattern and may explain the low early marine survival probabilities measured over less than two weeks. Healthy populations of marine mammals in the Salish Sea have the potential to consume a large proportion of steelhead migrants, and may account for the high rates of mortality sustained in Puget Sound. Puget Sound also supports populations of several seabirds capable of consuming a 150-200 mm smolt (e.g., cormorants, Caspian terns, loons: *Gavia sp.*, common murres: *Uria aalge*), but none of these species have dramatically increased in abundance since the 1980's, when steelhead populations began to decline.

The present study supports the general understanding that anadromous salmonid mortality rates during the early marine period exceed those during later periods when fish are larger and in different environments. Data from this analysis suggest that wild steelhead smolts experience early marine survival rates from river mouth to Strait of Juan de Fuca between 0.8% to 39.3%, with average travel times ranging from 6.2 to 18.1 days. Using an instantaneous mortality rate (based on estimated early marine survival rates and average population-specific travel times; -ln (survival probability$_{RM\text{-}JDF}$)/average migration time$_{RM\text{-}JDF}$) we can project forward the percentage of smolts remaining after only one month at sea (range 0% - 3.2%). Mortality rates after open ocean entry must decrease then, for there to be any adult steelhead returns. Understanding the specific mechanisms causing high early marine mortality rates of Puget Sound steelhead trout populations could be critical in predicting their viability over the long term and identifying management measures to improve the status of populations.

For details, see publication: Moore et al. 2015. Multi-population analysis of Puget Sound steelhead survival and migration behavior. *Marine Ecology Progress Series*, 537 (217-232). DOI: 10.3354/meps11460.

Figure 1. Average freshwater (PR-RM; black bars) and early marine (RM-JDF; light bars) survival probabilities ± SE for all Puget Sound (A) and Hood Canal (B) wild and hatchery-tagged steelhead populations.



Study 2: Western Washington State steelhead trout (*Oncorhynchus mykiss*) spawner abundance and marine survival trends

Neala W. Kendall*, Gary Marston, and Matthew Klungle

Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501-1091 USA

*email: Neala.Kendall@dfw.wa.gov

Pacific salmon and trout (*Oncorhynchus* sp.) populations form diverse complexes around the Pacific Rim and differ in productivity. This productivity can be impacted by a range of factors, including anthropogenic activities and natural variations in their environment, including large-scale climate effects and localized environmental conditions. Monitoring population abundance and survival trends over space and time is essential for identification of environmental drivers and other factors affecting survival and population dynamics along with appropriate management actions. Such monitoring includes evaluating trends in population abundance, productivity, and survival; whether and how these trends differ among neighboring regions; and the spatial and temporal co-variation of these trends. With this information we can better understand the drivers of these trends, the stability and resilience of the populations, forecast future run sizes, focus conservation efforts, and identify potential management actions.

Steelhead trout (*O. mykiss*) populations have declined in abundance over time throughout their range. Together with their resident counterparts (rainbow trout, the non-migratory form of *O. mykiss*), they are an important part of the ecosystem, economy, and culture of the Pacific Northwest. Steelhead trout are often monitored less than other salmonids due to their complex life history, which differs from other Pacific salmon. While marine survival rates of all five species of Pacific salmon have been examined by numerous papers, such an analysis has been done for only one steelhead population—the Keogh River of British Columbia, Canada. Studies tracking the marine survival of Pacific salmon populations have documented mostly positive correlations across the North Pacific Ocean, demonstrating regional coherence, and that populations that are closer to each other geographically are more tightly correlated, indicating local coherence. Given the differing life history and marine distribution of steelhead trout, their survival data may or may not show similar patterns.

Here we document Washington State steelhead trout population abundance and marine survival trends and examine their synchrony. These fish were produced either by natural spawning (termed "wild") or in hatcheries. We first present western Washington wild steelhead total run (catch plus spawning escapement) size trends from 27 populations within four Distinct Population Segments (DPSs) since 1980. We then estimate wild (12 populations or sub-populations) and hatchery (30 stocks) steelhead smolt-to-adult return (SAR) rates since the 1970s. SAR rates represent the percent of smolts that survive from freshwater to the ocean and back to freshwater. We then estimate spatial and temporal covariation in these trends.

We first gathered yearly total run size data since 1980 for wild fish, which is equal to the number of spawners plus the number of fish caught, from Washington Department of Fish and Wildlife's (WDFW) run reconstruction reports and SCoRE database (https://fortress.wa.gov/dfw/score/score/). Annual total run sizes ranged from < 50 fish in the Upper Gorge population of the Lower Columbia River DPS to > 20,000 fish in the Quillayute River system of the Olympic Peninsula DPS (Table 1). Abundance values varied greatly over time (Figure 1), though many populations have showed a declining trend.

Using WDFW run reconstruction reports (R. Leland, WDFW, unpublished data), spawning ground data via the SCoRE database, smolt trap data (WDFW, unpublished data), and hatchery databases (WDFW, 2015, FishBooks hatchery database and Hatcheries Headquarters Database), we also gathered the data necessary to estimate SAR rates. Specifically, for each wild population or hatchery stock this included the annual number of wild or hatchery (usually marked by an adipose fin clip) smolts outmigrating to the ocean (S), the number of adults (spawning in natural environments for wild populations or returning to hatcheries for hatchery stocks; N), the number of wild or hatchery adult fish caught (C), and the age composition of the adults. Hatchery smolt releases were counted at each hatchery while wild smolts are counted at smolt traps. With the adult age data, we assigned the adults to a given outmigration year cohort (i). We compared these data to the number of smolts from that outmigration year cohort (S_i) to estimate the SAR for that cohort:

Eq. 1 $$SAR_i = \frac{N_i + C_i}{S_i}.$$

SAR rates have ranged from < 0.1% to 28% (Table 2) and have varied greatly over time and among populations (Figure 2). Most populations have shown a declining trend in SAR rates.

We used the MARSS (Multivariate Auto-Regressive State-Space) model package in the program R to examine variation in abundance and SAR trends among the steelhead populations. This model examines which population groups' trends (e.g., all populations in one big grouping with similar dynamics versus multiple groupings) are best supported by the data. For the total run data, we examined whether one big grouping containing all populations, three groupings (Puget Sound, Washington coast, and Lower Columbia River), seven groupings (four Puget Sound basins, north coast, south coast, and Lower Columbia), or eight groupings (four Puget Sound basins, north coast, south coast, and Lower Columbia broken into two groups) were better supported by the available data (Table 1). For the SAR data, we examined whether one, two, three, four, five, or six groupings were better supported (Table 2). We tested, and then confirmed, that both the process and observation error variance-covariance matrix structure were diagonal and equal, so they were the same for each grouping along the diagonal axis, for the whole time series there was a zero average rate of SAR change, and a mean-reverting state fit the data better than a random walk. Best models were identified by the lowest AIC_c scores.

For total run size, the model that fit the data best divided the populations into eight groupings (Table 1 and Figure 1). This suggests that we see varying population dynamics among geographical regions of western Washington State. Different drivers may be influencing population dynamics in these areas, both in freshwater and the ocean.

Western Washington State steelhead SARs were best divided into four groupings (Table 2 and Figure 2), with r steelhead from the lower Columbia River, the Washington coast, Puget Sound, and the Strait of Juan de Fuca each grouping separately. SAR trends have declined over time and the declines occurred at different times for the different groupings. While Washington coast and Lower Columbia populations'/stocks' SARs have recovered over the last decade, Puget Sound values have not. Strait of Juan de Fuca SARs have varied greatly over time and show less of an increasing or decreasing trend over time. Thus, different saltwater conditions may be influencing steelhead that originate from these different regions. Specifically, early ocean factors may be important given that all steelhead are thought to migrate up to the Gulf of Alaska in their first winter and then west towards Asia. Our results help to guide future work examining which environmental factors are most closely related to steelhead trout marine survival patterns.

Table 1. Adult total run size data for each steelhead population included in this study. Each population's DPS and geographical area is listed along with the MARSS model groupings and their ΔAIC_c values. The model with eight groupings was found to fit the data best (lowest ΔAIC_c score).

Population	DPS	Geographic region	Minimum total run	Maximum total run	Average total run	1 grouping	3 groupings	7 groupings	8 groupings
						124.8	16.5	14.1	0.0
Samish R. and Bellingham Bay winter	Puget Sound	Rosario Basin	125	1,028	675	1	1	1	1
Skagit R. summer and winter	Puget Sound	Whidbey Basin	2,629	15,933	7,955	1	1	2	2
Snohomish R. system winter	Puget Sound	Whidbey Basin	1,723	9,828	5,027	1	1	2	2
Stilliguamish R. winter	Puget Sound	Whidbey Basin	144	4,398	1,141	1	1	2	2
Green R. winter	Puget Sound	Central Sound	312	3,500	2,082	1	1	3	3
Puyallup/Carbon R. winter	Puget Sound	Central Sound	162	2,469	917	1	1	3	3
White R. winter	Puget Sound	Central Sound	205	1,762	638	1	1	3	3
Nisqually R. winter	Puget Sound	South Sound	198	6,671	1,491	1	1	4	4
Hoh R. winter	Olympic Peninsula	North Coast	2,541	5,783	4,353	1	2	5	5
Queets system winter	Olympic Peninsula	North Coast	4,883	12,060	7,665	1	2	5	5
Quillayute system winter	Olympic Peninsula	North Coast	6,786	21,615	14,165	1	2	5	5
Quinault system winter	Olympic Peninsula	North Coast	3,524	7,944	5,395	1	2	5	5
Chehalis system winter	Southwest Washing	South Coast	6,299	19,051	10,385	1	2	6	6
Humptulips R. winter	Southwest Washing	South Coast	1,181	7,125	3,024	1	2	6	6
Willapa system winter	Southwest Washing	South Coast	1,835	11,547	3,776	1	2	6	6
Grays R. winter	Southwest Washing	Outer lower Columbia	158	1,224	720	1	3	7	7
Mill, Abernathy, and Germany Cr. winter	Southwest Washing	Outer lower Columbia	83	528	329	1	3	7	7
Skamokowa-Elochoman R. winter	Southwest Washing	Outer lower Columbia	192	784	522	1	3	7	7
Coweeman R. winter	Lower Columbia	Inner lower Columbia	108	1,088	484	1	3	7	8
East Fork Lewis R. summer	Lower Columbia	Inner lower Columbia	139	1,084	531	1	3	7	8
Kalama R. summer	Lower Columbia	Inner lower Columbia	140	2,926	720	1	3	7	8
Kalama R. winter	Lower Columbia	Inner lower Columbia	396	2,400	1,094	1	3	7	8
South Fork Toutle R. winter	Lower Columbia	Inner lower Columbia	210	2,222	868	1	3	7	8
Upper Gorge winter	Lower Columbia	Inner lower Columbia	7	53	25	1	3	7	8
Washougal R. summer	Lower Columbia	Inner lower Columbia	103	842	367	1	3	7	8
Washougal R. winter	Lower Columbia	Inner lower Columbia	92	1,114	369	1	3	7	8
Wind R. summer	Lower Columbia	Inner lower Columbia	192	1,468	641	1	3	7	8

Table 2. Smolt marine survival values for each steelhead hatchery stock or wild population included in this study. Each stock or population's run type (summer vs. winter), origin (hatchery vs. wild), geographical region, and the ocean entry year (OEY) range with data available is given along with the MARSS model groupings assessed and their ΔAIC_c values. LC = Lower Columbia, PS = Puget Sound, and SJdF = Strait of Juan de Fuca. Blank values in the groupings indicate that an insufficient time series was available to include the population in the MARSS modeling.

Stock or population	Run	Origin	Geographic region	OEY	Minimum SAR	Maximum SAR	Average SAR	Panmictic (1 grouping)	Coast+LC, PS, SJdF (3 groupings)	Coast, LC, PS+SJdF (3 groupings)	Coast, LC, PS, SJdF (4 groupings)
							AIC_c:	143.1	40.3	5.0	0
Big Beef Creek	winter	wild	Puget Sound	2005-2010	0.6%	5.3%	2.0%				
Bingham Creek	winter	wild	Coast	1996-2009	3.2%	19.8%	8.8%	1	1	1	1
Chehalis River	winter	hatchery	Coast	1981-2012	0.8%	4.6%	2.0%	1	1	1	1
Coweeman River	winter	wild	Lower Columbia	2005-2008	1.3%	5.0%	3.3%				
Cowlitz River	summer	hatchery	Lower Columbia	1993-2008	0.9%	6.7%	3.1%	1	1	2	2
Cowlitz River	winter	hatchery	Lower Columbia	1995-2010	0.6%	4.4%	1.8%	1	1	2	2
Cowlitz River	late winter	hatchery	Lower Columbia	1999-2010	0.6%	4.4%	1.9%				
Cowlitz River	winter total	hatchery	Lower Columbia	1993-2010	0.5%	3.8%	1.7%	1	1	2	2
Deep Creek	winter	wild	Strait of Juan de Fuca	1998-2007	3.0%	6.5%	4.6%				
East Twin	winter	wild	Strait of Juan de Fuca	2001-2007	0.8%	9.9%	4.4%				
Elochoman River	winter	hatchery	Lower Columbia	1993-2010	0.7%	3.0%	1.3%	1	1	2	2
Elwha River	winter	hatchery	Strait of Juan de Fuca	1985-2001	0.5%	10.3%	2.2%	1	3	3	4
Grays River	winter	hatchery	Lower Columbia	1998-2010	1.1%	3.6%	2.0%				
Green River	summer	hatchery	Puget Sound	1993-2011	0.2%	1.8%	0.8%	1	2	3	3
Green River	winter	hatchery	Puget Sound	1982-2010	0.2%	8.8%	1.5%	1	2	3	3
Humptulips River	summer	hatchery	Coast	1995-2008	0.5%	3.7%	1.3%				
Humptulips River	winter	hatchery	Coast	1977-2012	0.2%	4.4%	1.5%	1	1	1	1
Kalama River	summer	hatchery	Lower Columbia	1998-2009	2.8%	17.9%	6.9%				
Kalama River	winter	hatchery	Lower Columbia	1992-2010	0.4%	6.2%	3.4%	1	1	2	2
Kalama River	winter and summer	wild	Lower Columbia	1978-2009	3.4%	16.0%	8.5%	1	1	2	2
Lewis River	summer	hatchery	Lower Columbia	1994-2009	1.0%	6.5%	3.7%	1	1	2	2
Lewis River	winter	hatchery	Lower Columbia	1993-2010	0.3%	4.0%	2.0%	1	1	2	2
Nisqually River	winter	wild	Puget Sound	2009-2011	0.4%	0.8%	0.5%				
Nooksack River	winter	hatchery	Puget Sound	1999-2011	0.1%	1.5%	0.5%				
Puyallup River	winter	hatchery	Puget Sound	1984-2006	0.1%	4.0%	0.7%	1	2	3	3
Queets River	winter	wild	Coast	1981-2007	4.0%	20.7%	11.9%	1	1	1	1
Quillayute River	summer	hatchery	Coast	1999-2011	3.7%	10.6%	5.9%				
Quillayute River	winter	hatchery	Coast	1982-2011	1.4%	27.5%	7.2%	1	1	1	1
Salt Creek	winter	wild	Strait of Juan de Fuca	2000-2007	1.7%	10.1%	6.5%				
Samish River	winter	hatchery	Puget Sound	1977-1979	0.9%	3.0%	2.0%				
Skagit River	winter	hatchery	Puget Sound	1982-2010	0.1%	3.7%	1.0%	1	2	3	3
Snohomish River	summer	hatchery	Puget Sound	1994-2011	0.7%	3.2%	1.8%	1	2	3	3
Snohomish River	winter	hatchery	Puget Sound	1986-2010	0.6%	7.6%	2.0%	1	2	3	3
Snow Creek	winter	wild	Strait of Juan de Fuca	1978-2011	0.5%	19.6%	4.9%	1	3	3	4
Stillaguamish River	summer	hatchery	Puget Sound	1996-2011	0.1%	4.7%	0.7%	1	2	3	3
Stillaguamish River	winter	hatchery	Puget Sound	1994-2010	0.1%	1.6%	0.6%	1	2	3	3
Washougal River	summer	hatchery	Lower Columbia	1993-2009	0.7%	7.4%	3.4%	1	1	2	2
Washougal River	winter	hatchery	Lower Columbia	1994-2010	0.2%	3.2%	1.3%	1	1	2	2
West Twin	winter	wild	Strait of Juan de Fuca	2001-2007	0.9%	12.0%	4.8%				
Willapa River	winter	hatchery	Coast	1994-2010	0.2%	3.5%	1.7%	1	1	1	1
Wind River	summer	wild	Lower Columbia	2003-2011	1.7%	7.6%	4.1%				
Wynoochee River	summer	hatchery	Coast	1994-2009	1.3%	3.5%	2.1%	1	1	1	1

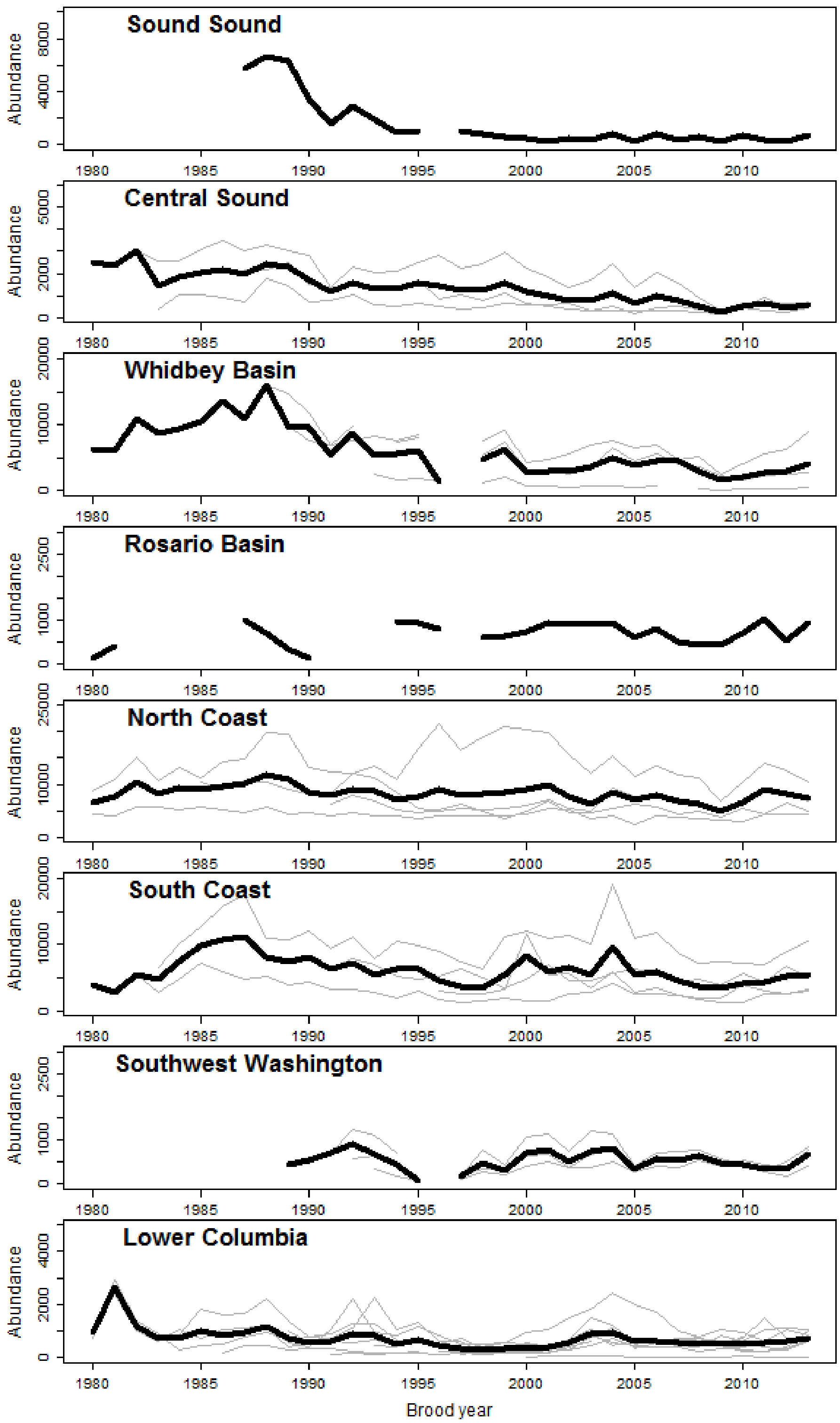


Figure 1. Total run size of Western Washington State steelhead trout. Based on MARSS model analysis of the trends, populations are divided into eight groupings. Thin grey lines are individual population trends while thick black lines are average values in each grouping.

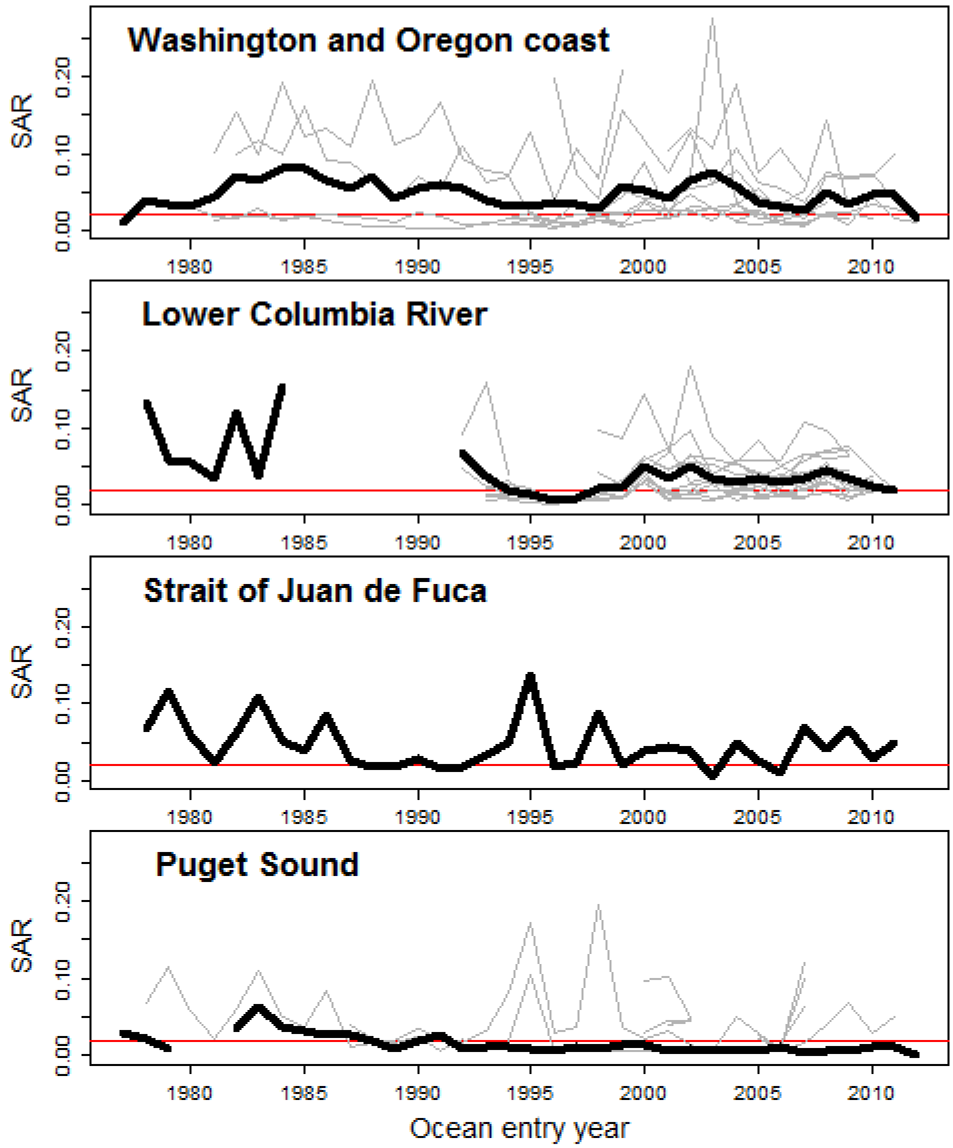


Figure 2. SAR rates in the ocean of Western Washington State steelhead trout. Based on MARSS model analysis of the trends, populations/stocks are divided into four groupings. Thin lines are individual population/stock SARs while thick lines are average values in each region. The thin red line at 0.02 is presented to facilitate comparison among regions.

Study 3: Fish characteristics and environmental variables related to marine survival of Western Washington State steelhead trout (*Oncorhynchus mykiss*)

Neala W. Kendall[1] and Correigh M. Greene[2]

[1]Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501-1091 USA

email: Neala.Kendall@dfw.wa.gov

[2]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration Fisheries, 2725 Montlake Boulevard E., Seattle, WA 98112-2907

Survival rates through the ocean migration of many populations of Western Washington steelhead trout (*Oncorhynchus mykiss*) have declined over the past 35 years, though survival trends have varied considerably over time and among populations. Such survival can be influenced by the condition of the fish as they exit freshwater along with environmental conditions they experience in their natal streams, the nearshore environment, and the open ocean. While a number of studies have examined variables related to marine survival of the five species of Pacific salmon (*Oncorhynchus* sp.), little work has been done on steelhead trout due to the paucity of marine survival data over space and time. By understanding the variables related to the marine survival trends, their spatial scale, and how their relationships with marine survival trends vary over time, we can better understand the stability and resilience of steelhead trout populations, forecast future run sizes, focus conservation efforts, and identify potential management actions.

Here we relate Washington State steelhead trout population marine survival trends, estimated as smolt-to-adult return (SAR) rates, to a variety of indicators, including those related to fish characteristics and the freshwater and marine environments (Table 1). The environmental variables vary in spatial scale from freshwater conditions of a population's natal steam to Puget Sound basin-specific variables to large-scale, ocean-wide indicators such as the PDO. We have SAR data (percent of smolts that survived from leaving freshwater to returning to spawn as adults) from 12 populations or sub-populations of steelhead trout that were produced by natural spawning (termed "wild") and 30 stocks of steelhead trout produced by hatcheries.

For variables with long-term data, we employed linear mixed effects models in the program R to examine their relationships with the SAR data. Because many of the variables are correlated with each other, we initially employed models with individual variables and examined which one best explained the SAR trends based on their AICc estimates. For variables for which long-term data were not available, we qualitatively examined correlations between these variables and the SAR data.

Preliminary analyses found that linear mixed-effects models including environmental variables generally had lower AICc values than models with fish characteristics. Of the environmental variables, lower Southern Oscillation Index (SOI) values, lower sea surface temperatures, increased coastal upwelling, higher adult herring abundance, fewer hatchery coho releases, and fewer harbor seals were found to be correlated with increased SAR rates for western Washington steelhead trout (Table 1). While a number of variables including smolt release date and size may be related to SARs, intra-annual variation may be an important driver and cannot be assessed with our preliminary model structures. Specifically, the response variable in our modeling structure, SAR, is estimated annually rather than intra-annually. For example, smolts migrating from freshwater to the ocean early in a season may do better than those outmigrating later in the year, but this variation in SARs is not reflected in our data. One variable that correlates strongly with SARs is harbor seal abundance (Figure 1), which has increased consistently over the time periods where declines in SARs have been seen for many wild steelhead populations and hatchery stocks. Further analysis will use updated SAR values and alternative modeling techniques such as multivariate analyses and principal component analyses to examine relationships between SARs and multiple fish characteristics and environmental conditions.

Table 1. Fish characteristics and environmental predictor variables used in the linear mixed effect modeling and qualitative analyses relating steelhead SARs to such variables, the spatial scale where the variables were measured, and the best-fit model results. “+” means that an increased value of the variable was related to higher SAR values while “-” means it was associated with lower SAR rates.

Predictor variable	Spatial scale	Relationship with SAR values
Fish characteristics		
Smolt count	Population/stock specific	
Hatchery broodstock type (Skamania, Chambers, native)	Stock specific	
Hatchery broodstock management (segregated vs. integrated)	Stock specific	
Percent of smolts from off-site hatchery	Stock specific	-
Smolt weight	Population/stock specific	
Smolt release start date	Stock specific	
Smolt release end date	Stock specific	
Environmental variables		
NOI	Pacific Ocean	
SOI	Pacific Ocean	-
Pacific coast sea surface temperature	Pacific Ocean	-
Sea surface temperature (coastal shelf)	Washington coast	
Coastal upwelling index (45N)	Washington coast	+
Spring transition	Washington coast	
Copepod community index	Washington coast	
Winter ichthyoplankton	Washington coast	
Chlorophyll a level	Washington coast	
Race Rocks temperature	Puget Sound	
Race Rocks salinity	Puget Sound	
Neah Bay sea level	Puget Sound	
Strait of Juan de Fuca sea surface salinity	Puget Sound	
Coastal upwelling index (48N)	Puget Sound	+
River flow	Population/stock specific	
Temperature	Puget Sound basin specific	
Salinity	Puget Sound basin specific	
Dissolved oxygen	Puget Sound basin specific	+
Chlorophyll a level	Puget Sound basin specific	
Density	Puget Sound basin specific	
pH	Puget Sound basin specific	
Light transmissivity	Puget Sound basin specific	
Adult herring abundance	Puget Sound basin specific	
Adult herring spawn timing	Puget Sound basin specific	
Hatchery coho abundance	Puget Sound basin specific	-
Seal abundance	Puget Sound basin specific	-
Marine bird abundance	Puget Sound basin specific	

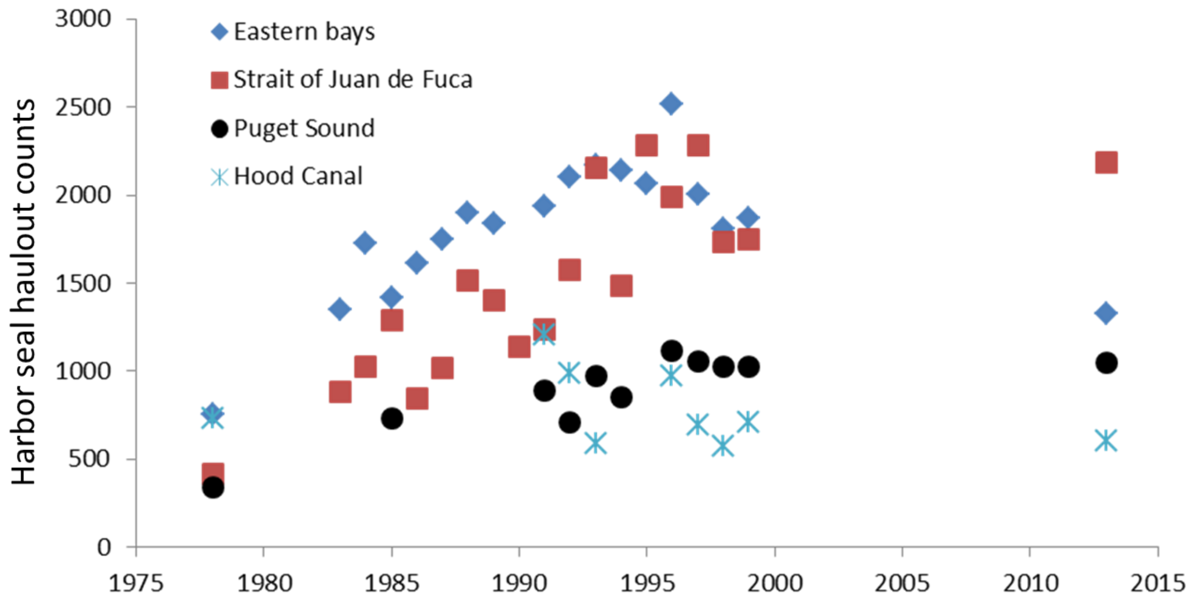


Figure 1. Southern Salish Sea harbor seal haulout count data from 1978-1999 and 2013. Data from Jeffries et al. (1999 Journal of Wildlife Management) and unpublished WDFW data.

Study 4: Geographic location outweighs effects of freshwater rearing and hatchery influence on early marine survival of Puget Sound steelhead

Megan E. Moore and Barry A. Berejikian

Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration Fisheries, P.O. Box 130, Manchester, 98353, USA

Multi-population studies conducted in both the Canadian and US waters of the Salish Sea have documented high rates of mortality as steelhead smolts migrate from freshwater to the open ocean, which may be driving concurrent population declines on both sides of the border. It is unknown whether the documented mortality is caused by freshwater processes that degrade the condition or fitness of smolts entering saltwater, or rather by more direct mechanisms within the marine environment. This study took advantage of contrasting conditions in geographically proximate river systems to test for the effects of freshwater rearing conditions and hatchery introgression on survival rates of steelhead migrating from river mouth to the Pacific Ocean. The Green/Duwamish River in the Central Puget Sound region of the Salish Sea is characterized by habitat conditions typically understood as unhealthy for salmon, while high quality salmon habitat dominates in the Nisqually River only about 60 kilometers to the south. Ongoing hatchery steelhead releases on the Green River began in 1964 and continue to present, while all Nisqually hatchery steelhead programs were terminated in 1994 (FishPlants Database, WDFW). We performed a reciprocal transplant experiment (i.e., cross-planting smolts from one river into another and vice versa) using steelhead smolts from the Green and Nisqually Rivers to determine whether low early marine survival rates could be caused by population-specific effects like freshwater rearing conditions or hatchery introgression, or if direct effects within the marine environment were more likely the cause.

Fifty smolts from each experimental group (Green Home, Green Away, Nisqually Home, and Nisqually away) were implanted with acoustic transmitters and released in their river of origin or transplanted. Survival probabilities were estimated using mark recapture models, populated with detections from telemetry receiver arrays deployed along the outmigration route (Green and Nisqually river mouths, Tacoma Narrows (NAR), Central Puget Sound (CPS), Admiralty Inlet (ADM), Strait of Juan de Fuca (JDF); Figure 1). Steelhead from the Green and Nisqually populations released at both home and away release locations survived at similar rates through all common migration segments. The probability of survival through freshwater (PR-RM) was high (91.2 ± 2.0%) relative to survival probability estimates through marine migration segments (Figure 2). The lowest survival probability was estimated for fish released from the Nisqually migrating from NAR to CPS (33.8 ± 7.0%), a segment not encountered by smolts entering Puget Sound from the more northern Green River mouth. Migration through this extra segment substantially decreased overall RM-JDF survival probability, with smolts released from the Nisqually River surviving at an estimated 5.9 ± 4.2% compared to an estimate of 17.4 ± 7.1% for Green River releases (Figure 2). Mark-recapture model comparison indicated that release date affected survival of smolts released in the Nisqually River, with early releases surviving better than later-released smolts.

Population, release location, translocation, and fork length factors had little effect on survival probabilities. Travel times from river mouth to the JDF line were short (Nisqually releases: 257 km in 9.80 ± 1.19 days; Green releases: 187 km in 8.80 ± 0.44 days), and travel times spent in each migration segment were similar among all four release groups.

Similar survival probabilities among smolts released in the Green and Nisqually rivers, despite clear differences in habitat and hatchery influence, render these factors unlikely to substantially influence early marine survival of these populations. The location of the river mouth within Puget Sound had the greatest bearing on survival of steelhead smolts through Puget Sound; smolts with shorter migration distances survived better than those with longer distances to migrate. Low survival rates combined with the short observed Puget Sound residence times suggest predation as a likely mechanism of mortality, but direct evidence of predation is lacking and there may be other factors involved (e.g., disease). Future research will focus on identifying sources of mortality that act in similar ways across populations within the marine environment.

Figure 1. Map of the Puget Sound region with telemetry receiver arrays (black circles) at the Green and Nisqually river mouths, the Tacoma Narrows (NAR), Central Puget Sound (CPS), Admiralty Inlet (ADM), and the Strait of Juan de Fuca (JDF). Black stars indicate the locations of steelhead smolt trapping sites, and black triangles depict release sites.

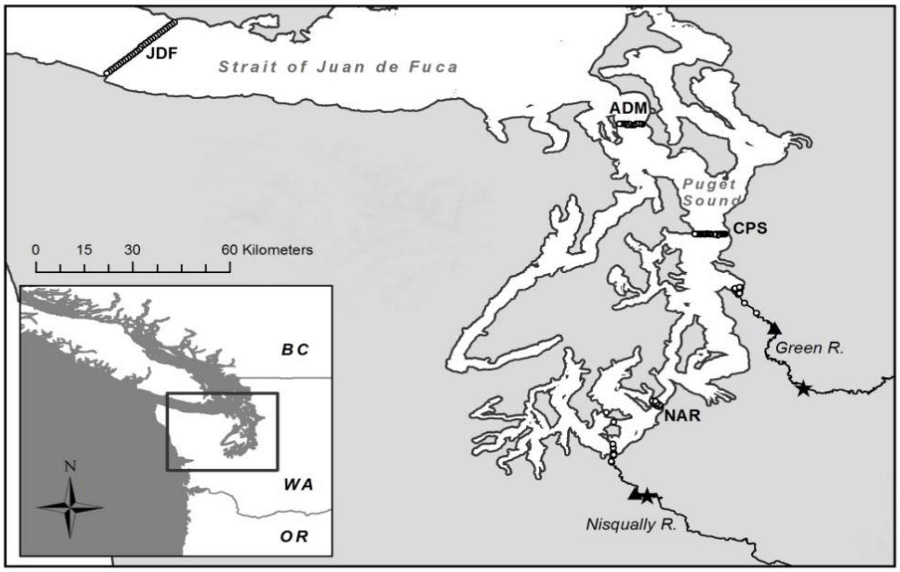
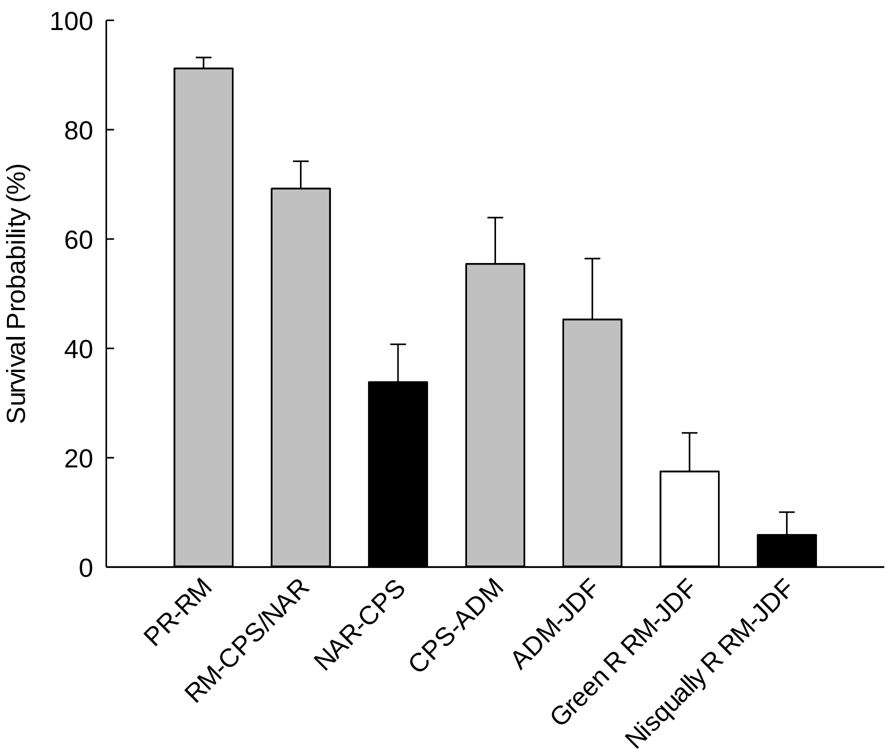


Figure 2. Survival probabilities ± standard errors through specific migration segments. Gray bars represent data for both populations, black bars refer to survival probabilities of smolts released in the Nisqually River, and white bars refer to survival probability of Green River released smolts.



Study 5: Steelhead smolt releases from Skagit River used to estimate detection efficiency of Strait of Juan de Fuca acoustic telemetry line

E.J. Connor[1], E. Jeanes[2], and C. Morello[2]

[1]Environmental Affairs Division, Seattle City Light, 700 Fifth Avenue, Seattle, WA 98124-4023

[2]R2 Resource Consultants, Inc., 15250 NE 95th Street Redmond, WA 98052

An experimental release of acoustically tagged steelhead smolts from the Skagit River was completed in the spring of 2014 to estimate the detection efficiency of the Strait of Juan de Fuca (SJDF) acoustic receiver line. This study was also conducted to identify the migratory routes of Skagit steelhead smolts through the Puget Sound, to compare smolt survival rates of among these routes. We surgically implanted acoustic transmitters (tags) in 100 steelhead smolts obtained at the WDFW Marblemount Hatchery in the upper Skagit River watershed. We tagged 50 of the smolts using Vemco V7-2L tags, which was the same type of tag used for tracking the marine migration survival of steelhead smolts released from the Green and Nisqually rivers in 2014. The remaining 50 smolts were tagged with larger and higher power Vemco V9-2H tags, which we determined from prior studies as having a 100% detection efficiency at the SJDF line. The SJDF line is the most important receiver array in the Puget Sound, since it is the largest and the last array that can detect tagged fish prior to entering the Pacific Ocean.

The tagged steelhead smolts were released into the Skagit from the Marblemount Hatchery on May 13, 2014. These fish migrated 127 km down the Skagit River from the release site to the Skagit Bay estuary in an average of 12 days. A total of 33 of the 100 tagged smolts were detected in Skagit Bay, indicating that freshwater survival rate over this distance was approximately 33%. We determined that the detection efficiency of the two receiver arrays deployed in north end and south end of Skagit Bay was close to 100% based upon a comparison of V7 and V9 detection rates. A total of 10 tagged Skagit steelhead smolts were detected at the SJDF line prior to their outmigration into the Pacific Ocean. Of these tags, four were V7-2L tags and six were V9-2H tags. Given that the detection efficiency of the V9-2H tags was 100%, the estimated detection efficiency of V7-2L tags at the SJDF line was 66.7%. The average travel time for the Skagit steelheads smolts from Skagit Bay to the SJDF line was five days, with the travel distance for these smolts averaging 163 km over two possible migration routes through the Puget Sound. The average marine survival rate for steelhead smolts (Skagit Bay to SJDF line) was approximately 33%, while the average combined freshwater and marine survival rate from the Marblemount Hatchery to SJDF line was approximately 12%.

We also compared the marine survival rates of Skagit steelhead smolts between two possible migration routes: 1) north through Deception Pass and then west to the Strait of Juan de Fuca for a travel distance of 119 km; and 2) south through Saratoga Passage along the east side of Camano Island and then northwest through Admiralty Inlet to the Strait of Juan de Fuca for a travel distance of 207 km. A total of 18 of the 33 tagged smolts detected in Skagit Bay migrated via the northern route, while 15 of the 33 smolts migrated via the southern route. This means that 55% of the smolts migrated the shorter northern route via Deception Pass, while 45% of the smolts migrated the longer southern route via Saratoga Passage and Admiralty Inlet. The estimated marine survival rate for Skagit steelhead smolts migrating the northern route was 39%, while the estimated marine survival rate for smolts migrating the southern route was approximately 23%. This finding suggests that marine mortality increases as migration distances increase, which is consistent with predation as the major source of mortality to steelhead smolts in the Puget Sound. The overall estimated survival rate of Skagit steelhead smolts migrating through the Puget Sound in 2014 was 36%.

Study 6: Identifying Potential Juvenile Steelhead Predators in the Marine Waters of the Salish Sea

Scott F. Pearson[1], Steven J. Jeffries[1], Monique M. Lance[1], and Austen Thomas[2,3]

[1]Wildlife Science Division, Washington Department of Fish and Wildlife, Olympia

[2]Zoology Department, University of British Columbia, Vancouver

[3]Molecular Genetics Laboratory, Washington Department of Fish and Wildlife, Olympia

Puget Sound wild steelhead were listed as threatened under the Endangered Species Act in 2007 and their populations are now less than 10% of their historic size. Data suggest that juvenile steelhead mortality is very high in the marine waters of the Salish Sea (waters of Puget Sound, the Strait of Juan de Fuca and the San Juan Islands as well as the waters surrounding British Columbia's Gulf Islands and the Strait of Georgia). Understanding the mechanism(s) responsible for low steelhead survival in the Salish Sea can help inform potential management solutions. One potential mechanism is top-down predation by fish-eating predators. To help us better understand the potential role of predators in steelhead decline, we identified possible marine mammal and bird predators of outmigrating juvenile steelhead based on predator distribution, abundance, and diet information. Given this review, we identified the next steps (research and information needs) for identifying and evaluating predation as a potential mechanism for low early marine steelhead survival.

Based on our literature review, we recommend that future research on the juvenile steelhead "predation hypothesis" focus on the diet, distribution and abundance of harbor seals, double-crested cormorants, Caspian terns, and Brandt's cormorants. In addition, although juvenile salmon have not been detected in stomach contents in Puget Sound, harbor porpoises have increased dramatically during the period of steelhead decline and, because they find their prey using echolocation, have a unique ability to exploit a resource like juvenile steelhead that tend to move individually or in small groups rather than in large schools. Finally, if additional resources are available, we would also include California sea lions and common murres. We recommend that research on this suite of potential predators be focused on gaining a better understanding of predator space use, foraging areas, and diet composition in areas of apparently high juvenile steelhead mortality (Hood Canal bridge area, Admiralty Inlet, and Central Puget Sound). All of these fish-eating species identified for additional research have demonstrated relatively stable or increasing population trends in recent years and their diet includes juvenile salmon, even if only a very minor component. To help us narrow the list of potential predators, we recommend initial surveys to assess relative predator abundance in areas of high steelhead mortality during the steelhead outmigration window – a period when we have poor information on predator abundance and distribution. One approach for assessing predator diet is to use new molecular techniques in combination with traditional techniques (hard part analysis) to help us understand the importance of steelhead to predator diet. This multiple predator approach has advantages in that it may not be a single predator that is contributing to low steelhead survival. If predation is identified as a factor contributing to steelhead declines, it is also important to gain a better understanding of potential ultimate factors that may be leading to high predation rates such as steelhead physical condition, potential hatchery effects, and human environmental modifications such as the Hood Canal Bridge. For details, see technical report: Pearson et al. 2015. Identifying potential juvenile steelhead predators in the marine waters of the Salish Sea. Salish Sea Marine Survival Project Technical Report. Washington Department of Fish and Wildlife, Wildlife Science Division, Olympia, WA @ marinesurvivalproject.com.

Table 1. Mammals and birds from Gaydos and Pearson (2011) that are relatively abundant in central and northern Puget Sound in the spring and summer and are fish eaters (piscivorous). We reviewed the literature to assess: 1) the degree of size overlap between fish in the diet and the size of outmigrating steelhead, 2) any evidence that the predator eats juvenile salmon and/or steelhead, and 3) and evidence that the predator eats juvenile steelhead. The species highlighted in green eat fish the size of outmigrating steelhead.

Common name	Scientific name	Diet overlap[1]	Eat Juvenile salmon or steelhead?[2]	Eat Juvenile steelhead?[2]
Mammals				
Harbor porpoise	*Phocoena phocoena*	Yes	No evidence	No evidence
Dall's porpoise	*Phocoenoides dalli*	Yes	No evidence	No evidence
Harbor seal	*Phoca vitulina*	Yes	Yes	Yes
California sea lion	*Zalophus californianus*	Yes	Yes	Yes
Birds				
Common loon	*Gavia immer*	Yes	?	?
Pacific loon	*Gavia pacifica*	Likely	Yes	?
Red-throated loon	*Gavia stellata*	Yes	?	?
Western grebe	*Aechmophorus occidentalis*	No	?	?
Red-necked grebe	*Podiceps grisegena*	Little	?	?
Horned grebe	*Podiceps auritus*	No	?	?
Double-crested cormorant	*Phalacrocorax auritus*	Yes	Yes	Yes (no local evidence)
Brandt's cormorant	*Phalacrocorax penicillatus*	Yes	Yes	?
Pelagic cormorant	*Phalacrocorax pelagicus*	Yes	?	?
Red-breasted merganser	*Mergus serrator*	Unlikely	Yes	?
Glaucous-winged/Western gull complex	*Larus glaucescens, L. occidentalis, and L. glaucescens x L. occidentalis*	Likely	Yes	Yes (no local evidence)
Caspian tern	*Sterna caspia*	Yes	Yes (estuary)	Yes
Common murre	*Uria aalge*	Moderate	Yes	?
Rhinoceros auklet	*Cerorhinca monocerata*	Little	Yes	No evidence
Pigeon guillemot	*Cepphus columba*	Little	No evidence	No evidence
Marbled murrelet	*Brachyramphus marmoratus*	No	Yes (freshwater)	?

[1]Yes = literature indicates that the predator regularly eats fish the size of juvenile steelhead; No = only eats fish smaller that juvenile steelhead; likely = little or no information on fish length in diet but based on the size of fish consumed by a similar sized congeneric, it is likely that they eat appropriate sized fish; Little = only the longest fish consumed overlap with the smallest juvenile steelhead; Moderate = approximately half of the fish consumed are similar to small to moderately sized juvenile steelhead.

[2]Yes = the literature indicates that they eat juvenile salmon and or steelhead; Yes (no local evidence) = documented to eat steelhead but there is no evidence from the Salish Sea despite considerable diet samples; No evidence = despite large sample sizes in the literature (100s of samples), there is no evidence that the species eats salmon/steelhead; ? = data are not adequate to evaluate this question.

Study 7: Predator-prey interactions between harbor seals and migrating steelhead smolts revealed by acoustic telemetry

B. A. Berejikian[1,*], M. E. Moore[1], and S. J. Jeffries[2]

[1]Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 7305 Beach Drive East, Port Orchard, WA 98366

[2]Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 9850

This study investigated predator-prey interactions between harbor seals and steelhead trout smolts and simultaneously tested the potential effect of the sound of acoustic telemetry transmitters on steelhead survival. Specifically, the study 1) described the degree of association between harbor seals and migrating steelhead smolts, 2) provided inferences of predation by harbor seals on steelhead smolts in Puget Sound, and 3) tested the effect of sound produced by acoustic telemetry tags on detection rates by seal-mounted telemetry receivers and survival of steelhead smolts. Steelhead trout smolts from the Green and Nisqually Rivers were implanted with acoustic telemetry transmitters (tags), and harbor seals in Central and North Puget Sound were outfitted with GPS tags and acoustic telemetry receivers capable of detecting the steelhead tags.

Detections of steelhead tags by harbor seals occurred both during and after the spring smolt outmigration period. A total of 6,846 tag detections from 44 different steelhead trout smolts (243 total tagged fish were released into two rivers) were recorded by the 11 seal-mounted receivers that were recovered. Central Puget Sound seal receivers detected a greater proportion of smolts surviving to the vicinity of the haulout locations (29 of 51; 58%) than Admiralty Inlet seals (7 of 50; 14%; $P < 0.001$). Detection data do not suggest that any of tagged smolts were consumed by the 11 monitored seals. Nine of the steelhead smolts were likely consumed by non-tagged harbor seals based partly on detections of stationary tags at the seal capture haulouts. Repeatable detection patterns of three tags over a three to four day period were consistent with an ingested tag present in the gut of a non-outfitted seal. We cannot rule out that some of the tags were deposited near these haulouts by other predators. Steelhead smolts implanted with transmitters that were silent for approximately two-thirds (10 days) of their migration before switching on had similar survival to those with continuously pinging tags, providing no evidence for effects of tag noise on the survival of steelhead smolts in Puget Sound. However, increasing the sample sizes would provide a more robust assessment and greater confidence in this result.

The present study provides the first data to suggest that harbor seals consume steelhead smolts in Puget Sound. We hypothesize that the Puget Sound ecosystem has changed such that steelhead smolts suffer greater predation rates under current conditions and that harbor seals contribute to mortality of migrating smolts. Depredations inferred from this type of data would need to be expanded to estimate a predation rate for areas not covered by the sampling design used in this study. For example, harbor seals are likely to defecate tags at locations other than monitored haulouts, and any stationary tags defecated away from the harbor seal tagging locations were not included in our inferred predation events. Additionally, the outfitted harbor seals in this study effectively monitored the Orchard Rocks, Blakely Rocks and Colvos Rocks haulouts, but these represent only a small portion of the harbor seal population in Puget Sound. Subsequent work will incorporate a greater number of moored acoustic receivers, mobile tracking to systematically search for stationary tags, broader spatial representation of the monitored harbor seal population, incorporation of diet data, and an analytical framework to estimate predation rates in Puget Sound.

For details, see publication: Berejikian et al. *in press*. Predator-prey interactions between harbor seals and migrating steelhead smolts revealed by acoustic telemetry. *Marine Ecology Progress Series.* 10.3354/meps11579.

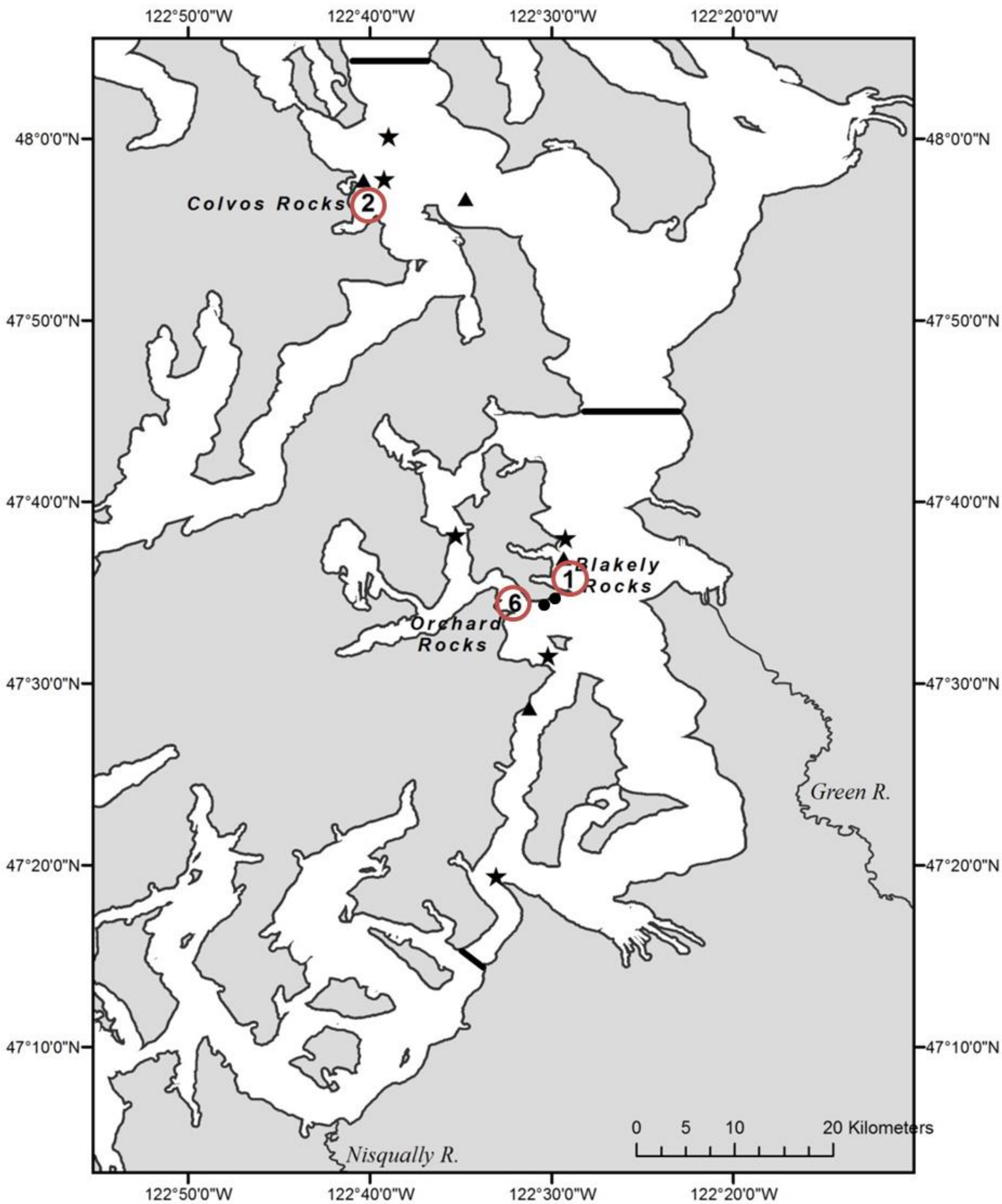


Fig. 5. Locations of steelhead smolts detected by harbor seals based on associations between a VMT detection and GPS location(s) ocurring less than 30 min apart (typically much less). Numbers of smolts detected at each haulout indicating mortalities are circled. Also shown are smolts i) detected by seal receivers which survived to a stationary array further along the migration route to the Pacific Ocean (▲), ii) detected after the smolt outmigration season but not later detected (★), or iii) associated with the Orchard Rocks haulout and later detected stationary nearby (●).

Study 8: Prevalence and load of *Nanophyetus salmincola* infection in outmigrating steelhead trout from five Puget Sound rivers

M.F. Chen[1], B.A. Stewart[1], K. Peabody[2], K. Snekvik[3] and P. Hershberger[4]

[1]Northwest Indian Fisheries Commission, 6370 Martin Way E., Olympia, WA 98670

[2]Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501

[3]Washington Animal Disease Diagnostic Laboratory, PO Box 647034 , Washington State University, Pullman, WA 99164

[4]United States Geologic Survey – Western Fisheries Research Center, Marrowstone Marine Field Station, 616 Marrowstone Point Rd., Nordland, WA 98358

Nanophyetus salmincola is a parasitic trematode, or flatworm, that infects salmonid fishes in the Pacific Northwest, including Washington, Oregon, and portions of California. The adult worm lives in the intestine of fish-eating birds and mammals. Eggs shed into the water hatch into miracidia which penetrate the first intermediate host, one of two species of snail *Juga plicifera* or *J. silicula*. Asexual reproduction occurs within the snail. Free-swimming cercaria are released from the snail and penetrate the secondary intermediate host, often a salmonid fish, in fresh and brackish water. The cercaria encyst as metacercaria in various organs of the fish, including gills, muscle and heart, but favor the posterior kidney. Penetration and migration by the cercaria through the fish causes damage to nearly every organ system. Once encysted, metacercaria survive the ocean phase of salmonid life cycle. *N. salmincola* is a likely contributor to mortality of juvenile coho salmon (*Oncorhynchus kisutch*) during the early ocean rearing phase, and it is the most prevalent pathogen of outmigrating steelhead in the estuaries of the Pacific Northwest.

A field survey was implemented from March – June 2014 to compare the prevalence and parasite load of *N. salmincola* infections in outmigrating steelhead from five Puget Sound watersheds and to assess changes in infection levels that occurred during the smolt out-migration through each watershed. *N. salmincola* infection prevalence and parasite loads were determined by counting metacercaria in posterior kidney samples. Tissue samples were collected and examined by standard histological methods.

The prevalence and parasite load of *Nanophyetus salmincola* were significantly higher in outmigrating steelhead smolts from central and south Puget Sound watersheds than in those from the north Sound, where infections were rarely detected (Table 1). In north Puget Sound, *N. salmincola* metacercaria were not detected in the kidneys of any outmigrating (in-river and estuary) hatchery or wild steelhead from the Skagit River (n=21) or Snohomish River watersheds (n=7); however 7.1% (3/42) were infected in adjacent North Puget Sound offshore areas, and one steelhead captured in the Skagit River (in-river) showed signs of *N. salmincola* on its gill but not in its kidney. Similarly, *N. salmincola* was not found in any smolts (n=24) from the Tahuya River in the Hood Canal watershed. The Green-Duwamish and Nisqually Rivers had high prevalence (73.2% and 98.6% respectively), and fish from these rivers also exhibited gill and heart pathology not found in the other three rivers. Each fish with histiocytic branchitis (gill inflammation) and/or histiocytic myocarditis (inflammation of the heart muscle) was found to have *N. salmincola* cysts in the respective organs (Table 1). The prevalence of *N. salmincola* increased in wild steelhead as they outmigrated from the Green-Duwamish River watershed, from 13.3% at the in-river sampling location, 86.7% at the estuary, and 100% in adjacent offshore areas of Puget Sound (Table 2). An analogous down-stream progression occurred among hatchery-origin steelhead. Further evidence for a lower watershed zone of *N. salmincola* was provided by samples from the Soos Creek hatchery (located on the lower reaches of the Green-Duwamish River watershed), where 100% infection prevalence (n=30) and high mean parasite loads (3800 metacercaria / posterior kidney) occurred after rearing steelhead smolts on surface water for 16 months; these fish were not released into the watershed. Similarly, *N. salmincola* occurred at high prevalence and parasite load among wild steelhead from the south Puget Sound (Nisqually River) watershed (Table 2), where 98-100% were infected at both the in-river location and the estuary, with high mean parasite loads (1798 – 2544 metacercaria/posterior kidney) occurring at both locations. The lower prevalence of gill/heart pathology (Table 1) and lower *N. salmincola* load (Table 2) of offshore fish as compared to fish still in the river, suggest that heavily or newly infected fish do not survive passage through Puget Sound.

A laboratory study did not provide any indication that heavy *N. salmincola* loads influenced the ability of out-migrating steelhead to survive seawater transition in a protected environment. However, the logistics of the laboratory study resulted in a lag of three weeks between *N. salmincola* exposure and seawater challenge, with the experimental seawater transition occurring after the most pathogenic stages of infection.

Although this study was not designed to determine cause-and-effect relationships between *N. salmincola* infections and steelhead survival, several lines of evidence support the observed trends in early marine survival throughout Puget Sound. For example, areas with the highest early marine mortality rates (see Study 1) in Puget Sound include the watersheds with high *N. salmincola* infection prevalence and parasite load. The mean parasite loads occurring in these watersheds were at levels previously reported to result in health effects and lower marine survival. Hatchery and wild steelhead both had heavy parasite burdens; however lower SAR’s among hatchery cohorts may be associated with their prolonged residence in lower-river/estuarine habitats, where additional exposures are likely to occur. Heart and gill pathology found in fish from the South and Central Sound rivers and estuaries suggests these fish may be compromised in their ability to swim. An increased predation risk likely results from the reduced swimming performance of infected cohorts, and this risk is likely heightened by the increased abundance of certain marine mammal predators in recent years. Because restoration options are currently being explored for the recovery of endangered Puget Sound steelhead stocks, it is recommended that further efforts be employed to understand the distribution of the intermediate invertebrate host and the impacts of *N. salmincola* on their early marine survival.

Table 1. Prevalence of *Nanophyetus salmincola*, other parasites and organ pathology in steelhead smolts from five Puget Sound river basins in 2014. Wild and hatchery (if present) steelhead captured at in-river traps and estuaries are combined to form a composite sample for each river. Steelhead smolts captured offshore of the Skagit and Snohomish Rivers are combined into a Whidbey Basin sample and fish captured offshore of the Green-Duwamish and Nisqually Rivers are combined into a South Central Puget Sound sample. Gill pathology is defined as fibrosis and inflammation. Heart Pathology is defined as fibrosis and inflammation of the myocardium. Sample size = n. Not done = ND.

Sample Location	% Prevalance *N. salmincola* (n)	% Prevalence Kidney Myxosporean spp.[1] (n)	% Prevalence *Sanguinicola* spp.[2] (n)	% Prevalence Gill Pathology (n)	% Prevalence Heart Pathology (n)
Skagit River	4.7 (21)	40 (5)	0 (5)	0 (5)	0 (5)
Whidbey Basin offshore	7.1 (42)	35.5 (31)	3.2 (31)	0 (31)	3.2[3] (31)
Snohomish River	0 (7)	20 (5)	0 (5)	0 (5)	0 (5)
Tahuya River[4]	0 (30)	ND	ND	ND	ND
Green-Duwamish River	73.2 (112)	12.4 (89)	0 (89)	28.2 (89)	45.0 (89)
South Central Puget Sound offshore	93.9 (15)	35.7 (14)	14.3 (14)	7.1 (14)	28.5 (14)
Nisqually River	98.6 (69)	47.4 (59)	33.9 (59)	42.3 (59)	69 (59)

[1]Small multicellular parasites found in kidney tubules, unidentified as to species.

[2]Small trematodes living in blood vessels of the gills which use the same snail host as *N. salmincola.*

[3]One of 31 fish from Whidbey Basin exhibited heart pathology and heavy *N. salmincola* infection.

[4]Twenty-four steelhead and six coho salmon were sampled from the Tahuya Trap

Table 2. Progression of *Nanophyetus salmincola* infection as wild steelhead smolts outmigrated down the Green-Duwamish and Nisqually Rivers in 2014.

Sample Location	n	% Prevalence	Mean Parasite Load of Infected Fish
Green River Trap	30	13.3	698
Green/Duwamish Estuary	30	86.7	933
Green/Duwamish Offshore	6	100	209
Nisqually River Trap	40	97.5	1798
Nisqually River Estuary	30	100.0	2544
Nisqually Offshore	4	75.0	1448

Study 9: Toxic contaminant exposure in juvenile steelhead in Puget Sound

S. M. O'Neill[1], A. Carey[1], L.A. Niewolny[1], G. M. Ylitalo[2], and J.E. West

[1]Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501

email: Sandra.ONeill@dfw.wa.gov

[2] Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration Fisheries, 2725 Montlake Boulevard E., Seattle, WA 98112-2907

The abundance of wild Puget Sound steelhead trout has declined significantly since the mid-1980s and they were listed as threatened under the Endangered Species Act in 2007. Low survival rates of smolts entering in the marine environment have been identified as key factor in that decline and a barrier to recovery. However, it is not known whether the mortality is caused by freshwater processes that degrade the condition or fitness of smolts entering saltwater or by more direct mechanisms within the marine environment. Disease and toxic contaminant exposure, acting independently or synergistically, may affect the health of juvenile steelhead and their marine survival.

These factors could reduce marine survival directly if the effects were lethal, or indirectly by reducing the health of the fish and increasing their susceptibility to predation. For example, contaminant exposure can alter the immune system, either alone, or in conjunction with other stressors (e.g., parasites), increasing susceptibility to naturally occurring pathogens causing lethal diseases and leading to population level effects. Data on toxic contaminant exposure are lacking for juvenile steelhead originating from Puget Sound. However, juvenile Chinook salmon migrating from urban rivers and estuaries of central regions of Puget Sound are exposed to toxic contaminants, including man-made persistent organic pollutants (POPs), often at concentrations at which health effects occur.

The study was designed to determine (1) whether juvenile steelhead are exposed to toxic chemicals as they migrate from rivers into Puget Sound that could reduce their marine survival, and if so, (2) does exposure coincide with lower survival rates found in some Puget Sound rivers? This work was combined with study 8, to estimate the prevalence and parasite load of *N. salmincola* in juvenile steelhead during outmigration from representative watersheds. The results were then used to assess the potential effects of contaminant exposure and of *N. salmincola* infections on steelhead early marine survival. We tested the null hypothesis that juvenile steelhead collected from river, estuary, and offshore marine habitats in the north, central, and south regions of Puget Sound were uniformly exposed to contaminants. Our predicted alternative hypothesis was that steelhead in the developed central region of Puget Sound are exposed to higher toxic contaminant levels and are in poorer condition than fish collected from similar habitats in north and south regions of Puget Sound.

Toxic contaminant exposure to persistent organic pollutants (POPs) was measured in wild steelhead smolts collected from river and estuary habitats of the Skagit, Green/Duwamish, and Nisqually rivers (hereafter referred to as river systems) and the offshore habitat of the Whidbey, Central and South marine basins of Puget Sound. Steelhead were not captured at the estuary habitat of the Skagit River system, preventing a comparison among the three habitat types in the north, central, and south regions of Puget Sound. However, significant difference in contaminant exposure were not expected among river and estuary habitat types because tagging studies have shown that the fish travel between river and estuary habitats in just a few days. Thus comparison of POP concentrations in fish by habitat type was limited to river system (i.e. river and estuary habitat combined), offshore habitats of marine basins and north, central and south regions of Puget Sound (i.e. river system and associated offshore basin combined). Bile samples were also collected and analyzed for the presence of rapidly metabolized contaminants (or their metabolites) that can affect fish health but do not accumulate to high concentrations in whole body tissue samples, including polycyclic aromatic hydrocarbons (PAHs) and estrogenic chemical (ECs). However, due to insufficient bile volume, contaminants analyses was limited to a subset of the samples with sufficient volume of bile for analyses (n= 14 for PAH metabolites and 9 for ECs), limiting statistical comparisons among river systems, offshore habitats, and regions. Contaminant tissue residues were compared with published adverse effects thresholds, where available, to evaluate the potential health effects of contaminant exposure on juvenile steelhead marine survival.

Among the POPs evaluated in whole-body samples of steelhead, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and dichloro-diphenyl-trichloroethanes (DDTs) were detected in all samples with mean lipid-normalized concentrations of 1000, 480 and 270 ng/g lipid, respectively (Table 1). Chlordanes, hexachlorobenzene, and dieldrin were detected less frequently (83%, 72%, and 28% of the samples, respectively), at concentrations just above the limits of quantitation for these chemicals, and consequently, had much lower mean concentrations (58, 40, and 29 ng/g lipid, respectively). No other POPs were detected in the steelhead whole-body samples. Detailed results are only reported here for PCBs, PBDEs and DDTs.

PCB concentrations were elevated in steelhead collected from the central region of Puget Sound but not high enough to reject the null hypothesis that steelhead from north, central and south regions of Puget Sound were uniformly exposed to PCBs. PCB levels ranged from 290 – 3500 ng/g lipid and the highest mean concentrations were measured in steelhead collected from the central region of Puget Sound (1200 ng/g lipid), 20 – 25% higher than those measured in fish from north and south regions (910 and 960 ng/g lipid, Table 1), but not significantly different. Similar regional differences in PCB concentration were observed among river systems and among offshore marine basins (Table 1). Concentrations in fish from the Green/Duwamish were 26- 42% higher than in other river systems, and in fish in the Central Basin were 29% higher than other marine basins. Overall, mean lipid-normalized PCB concentrations were slightly higher in steelhead collected from marine basins (1200 ng/g lipid) than those from the river systems (902 ng/g lipid) because fish from the marine basins had slightly lower lipids levels than those in the river systems. Total PCB concentrations in steelhead were below concentrations known to cause adverse effects to the health of juvenile salmonids ($\geq$ 2400 ng/g lipid) in all samples collected from river systems, indicating that sub-lethal adverse effects from PCB contamination are not likely to occur in freshwater (Table 2). In contrast, total PCB levels exceeded harmful levels in 17-25% of samples from steelhead recovered from offshore habitats of the north, central and south regions of Puget Sound.

Surprisingly, PBDE concentrations were significantly elevated in steelhead from the south region of Puget Sound compared to the more developed central region. Similarly, mean levels of PBDEs (i.e., flame retardants) in steelhead from southern Puget Sound (920 ng/g lipid) were approximately three to five time times those measured in fish from central and north regions (290 and 190 ng/g lipid). Regional differences in PBDE concentration were also observed among river systems and among offshore marine basins (Table 1). Overall, PBDE levels did not vary by habitat type; steelhead from river systems and offshore marine habitats had similar PBDE concentrations (means of 550 and 400 ng/g lipid). In the south region of Puget Sound, 40% of the samples had concentrations high enough to cause increased disease susceptibility (Tables 1 and 2). In contrast, only 10% of the samples from the central region and none of the samples from north region of Puget Sound had concentrations high enough to potentially increased disease susceptibility.

Uniformly low DDT concentrations were detected in steelhead from all regions of Puget Sound, and we could not reject the null hypothesis for this contaminant. Specifically, levels ranged from 75 - 900 ng/g lipid (Table 1), and did not vary significantly among Puget Sound regions, among river systems, among marine basins, or among habitat types (i.e., river system vs. offshore marine basins). DDT concentrations were well below those known to adversely affect the health of juvenile salmonids (i.e., $\geq$ 6000 ng/g lipid, Table 2).

Total PCB levels exceeded harmful levels in 17-25% of samples from steelhead recovered from north, central and south offshore marine habitats, but none of the three rivers systems. The higher percentage of steelhead in offshore habitats with harmful PCB concentrations primarily is due to lower fish lipid content as migration proceeded. Health effects of PCB's are more likely to occur offshore rather than in-river or in estuaries. In contrast, PBDE (commonly referred to as flame retardants) concentrations exceeding the harmful threshold were found in 33% of Nisqually River steelhead samples from the river and estuary habitats. Unadjusted wet weight PBDE levels were also higher in the Nisqually than in the other two river systems, so this higher level of PBDE's was not due to lower lipid levels, lipid metabolism or residency in Puget Sound. PBDE levels were higher in steelhead from the Green-Duwamish than the Skagit river system, but harmful levels were not measured. Lower lipid levels in fish in offshore habitats resulted in 25% of Central Basin offshore fish with harmful PBDE levels, however as with the parasite results, northward migrating Nisqually River fish could be captured in other offshore areas.

The elevated levels of PBDE's and PCB's found in Puget Sound steelhead could cause loss of disease resistance, resulting in lower marine survival, particularly for populations from central and south regions of Puget Sound that have naturally high prevalence of N. salmincola. High concentrations of POP's have been found in juvenile Chinook salmon sampled from the more developed river, estuary and associated nearshore marine habitats of the central region of Puget Sound River. Several studies have documented that levels POP measured in Chinook salmon in these habitats are high enough to lower resistance to naturally occurring pathogen. Moreover, one study demonstrated the simultaneous exposure to PCB and the natural occurring parasite *N. salmincola,* caused lower resistance of juvenile Chinook salmon to the marine bacterial pathogen *Listonella anguillarum* than either stressor alone.

Metabolites of low and high molecular-weight PAHs were detected in bile of steelhead from all sites; for metabolites fluorescing at phenanthrene (PHN) wavelengths, concentrations ranged from to 2000 to 40,000 ng/mg bile protein. Levels of PAH metabolites were highest in steelhead from offshore marine habitat of the Central Basin (25,500 ng/mg bile protein), although samples sizes were insufficient to compare differences among river systems, among offshore habitats or among regions. Mean levels of biliary fluorescent aromatic compounds (FACs)-PHN in steelhead collected at all sites were at or above a threshold effect concentration of 2000 ng/mg protein for FACs-PHN for juvenile Chinook salmon linked to growth impairment, altered energetics, and reproductive effects.

Similarly high concentrations of PAH metabolites have been observed for juvenile Chinook salmon migrating through estuary and nearshore marine habitats of urban bays in Puget Sound. Unlike, juvenile Chinook salmon which spend several months feeding and rearing in estuary and nearshore marine habitats, juvenile steelhead migrate quickly through these habitats and do not appear to be actively feeding. Juvenile steelhead may be exposed in low PAHs in freshwater systems that are then concentrated in the bile during their outward migration when they are not actively feeding.

ECs were also detected in bile samples of steelhead but the small sample size limited interpretation of the results. Two naturally occurring estrogens, estradiol and estrone, which may come from exogenous sources, were detected at concentrations ranging from 3 – 12 and 2 – 6.3 ng/mL bile. Bisphenol A , a synthetic compound that mimics the natural estrogens was detected at concentrations ranging from 35-29,000 ng/mL bile.

In conclusion, although juvenile steelhead spend less time in the estuary than juvenile Chinook salmon, they accumulate PCB's and PBDE's during their longer freshwater residence, and due to lipid loss during migration, these contaminants reach biologically significant levels shortly after the fish enter the marine environment. Finding biologically significant levels of PBDE's in the relatively undeveloped Nisqually River watershed was unexpected and will be further pursued. At this point it uncertain to what extent exposure to PAHs may be influencing energy stores and growth rates in Puget Sound juvenile steelhead.

Table 1. Mean concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and dichloro-diphenyl-trichloroethanes (DDTs), measured in whole body samples of juvenile steelhead trout. Standard deviations of means are shown in parentheses.

Puget Sound Region	Sampling Location	Habitat Type	Sample size (n)	Lipid Content (%)	Lipid Weight POP Concentration (ng/g Lipid)		
					PCBs	PBDEs	DDTs
North	Skagit River	river	3[a]	1.0 (±0.15)	640 (±140)	130 (±38)	240 (±68)
		estuary	ns	-	-	-	-
		river + estuary	**3[a]**	**1.0 (±0.15)**	**640 (±140)**	**130 (±38)**	**240 (±68)**
	Whidbey Basin	**offshore**	**6[b]**	**0.95 (±1.0)**	**1000 (+710)**	**220 (+140)**	**360 (±280**
	Skagit River & Whidbey Basin	**All**	**9**	**0.97 (±0.83)**	**910 (±600)**	**190 (±120)**	**320 (±230)**
Central	Green/ Duwamish River	river	3[a]	1.2 (±0.29)	1100 (±90)	240 (±15)	230 (±22)
		estuary	3[a]	1.5 (±0.88)	1100 (±480)	250 (±130)	260 (±90)
		river + estuary	**6[a]**	**1.4 (±0.62)**	**1100 (±310)**	**240 (±84)**	**240 (±60)**
	Central Basin	**offshore**	**4[b]**	**1.0 (±0.37)**	**1400 (±710)**	**370 (±430)**	**310 (±350)**
	Green/ Duwamish River & Central Basin	**All**		**1.2 (±0.54)**	**1200 (±870)**	**290 (±260)**	**270 (±210)**
South	Nisqually River	river	3[a]	0.93 (±0.15)	740 (±370)	690 (±590)	260 (±150)
		estuary	3[a]	1.0 (±0.20)	880 (±200)	1400 (±1600)	160 (±38)
		river + estuary	**6[a]**	**0.97 (±0.16)**	**810 (±280)**	**1100 (±1100)**	**210 (±110)**
	South Basin	**offshore**	**4[b]**	**0.65 (±0.18)**	**1200 (±920)**	**700 (±260)**	**260 (±260)**
	Nisqually River & South Basin	**All**		**0.84 (±0.23)**	**960 (±920)**	**920 (±880)**	**230 (±180)**
All	All	All	29	1.0 (±0.58)	1000 (±600)	480 (±620)	270 (±200)

[a] each sample was composed of 4 -5 fish each

[b] individual fish sample

Table 2. Percentage of whole body steelhead samples exceeding persistent organic pollutants (POP) adverse effects thresholds for salmonids. Specific POP's assayed included polychlorinated biphenyls (PCB), dichloro-diphenyl-trichloroethanes (DDT) and polybrominated diphenyl ethers (PBDEs). Fish were not sampled (ns) from the Skagit River estuary habitat.

Region	Habitat	N	% samples > PCB effects threshold [a]	% samples > DDT effects threshold	% samples within range of PBDE levels associated with increased disease susceptibility [b]
Skagit River & Whidbey Basin	river	3[c]	0	0	0
	estuary	ns	-	-	-
	offshore	6[d]	17	0	0
	Total	9	11	0	0
Green/Duwamish River & Central Basin	river	3[c]	0	0	0
	estuary	3[c]	0	0	0
	offshore	4[d]	25	0	25
	Total	10	10	0	10
Nisqually River & South Basin	river	3[c]	0	0	33
	estuary	3[c]	0	0	33
	offshore	4[d]	25	0	50
	Total	10	10	0	40
	Overall Total	29	6.9	0	17

[a] 2400 ng/g lipid, Meador et al. 2002
[b] ≥ 470 ng/g lipid and ≤ 2500 ng/g lipid, derived from Arkoosh et al. 2013 and Arkoosh et al. 2010
[c] trap and estuary samples were analyzed as composites of 4-5 fish
[d] offshore samples were analyzed as individual fish

Study 10: Genome-wide association study of acoustically tagged steelhead smolts in the Salish Sea: measuring differences between survivors and non-survivors

Kenneth I. Warheit[1], Megan E. Moore[2], Barry A. Berejikian[2]

[1]Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501-1091 USA
email: kenneth.warheit@dfw.wa.gov

[2]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration Fisheries, P.O. Box 130, Manchester, 98353, USA

Genome-wide association studies (GWAS; Balding 2006, Bush and Moore 2012) use genome scans to document relationships between phenotypes (e.g., survival) and genotypes (e.g., individual single nucleotide polymorphisms (SNPs), blocks of linked SNPs, or genes), based on population samples. GWAS are used in a wide variety of studies ranging from understanding diseases in humans (e.g., McCarthy et al. 2008, Hindorff et al. 2009), improving the agricultural production of domestic animals and plants (e.g., Daetwyler et al. 2009, Purdie et al. 2011), to documenting specific behavior or morphology in wild animals (e.g., Johnston et al. 2011). In salmonids, GWAS have been used, for example, to gain a better understanding of developmental rates and migratory behavior in steelhead/rainbow trout (e.g., Miller et al. 2012, Hecht et al. 2013, Johnston et al. 2014), and disease resistance in Atlantic salmon (e.g., Houston et al. 2012). In this study, we looked for associations between genomic signatures in steelhead smolts and their survival while out-migrating through Puget Sound, Washington State.

As part of their studies on the migratory behavior and survival of steelhead smolts in Puget Sound, from 2006 through 2010, and in 2014, Moore and Berejikian (e.g., Moore et al. 2010) surgically implanted steelhead smolts with acoustic transmitters. The acoustic signal from each transmitter is unique and enables the identification and general location of individual fish when their signal is detected by a receiver. The position of acoustic receivers varied from year to year, but for the purpose of this study, we defined the fate (Puget Sound mortality or survivor) of each smolt using the detection of their transmitter at receivers located at Hood Canal Bridge, Central Puget Sound, Tacoma Narrows, Admiralty Inlet, and Strait of Juan de Fuca (Figure 1). Specially, a fish was defined as a survivor if it was detected leaving Puget Sound at the Strait of Juan de Fuca receiver array. To be identified as mortality, first the fish needed to be detected leaving the river from which it was tagged (i.e., entering the marine waters of Puget Sound), and then being undetected at any of the aforementioned receivers[26]. During the surgical procedure to implant the transmitters, a small fin clip was taken from each smolt for DNA analysis. We selected from an initial list of 881 fin clips from Big Beef Creek, and Dewatto, Duckabush, Hamma Hamma, Skokomish, Green, and Nisqually rivers, 288 samples for DNA sequencing (Figure 1; Table 1). We genotyped the fish using restriction-site associated DNA (RAD) sequences or RAD-tags

[26] For South Puget Sound populations undetected at NAR, CPS, ADM, and SJF; central Puget Sound populations, CPS, ADM, and SJF; and Hood Canal populations, HCB, ADM, and SJF. See Figure 1

(RAD-seq) (Miller et al. 2007, Baird et al. 2008, Davey et al. 2011). RAD-seq is a genome complexity reduction technique that sequences subsets of the genome that are adjacent to restriction enzyme recognition sites, and can characterize a genome-wide assessment of molecular diversity. Generally, RAD-seq can identify 1,000s to 10,000s SNPs. For this project we used the SbfI restriction enzyme. RAD-seq libraries were 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.

We used 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. We used two different sets of parameters in STACKS to establish two independent catalogs. We then selected RAD-tags that appeared in both catalogs, and aligned this combined and reduced catalog using the program BOWTIE 2 (Langmead and Salzberg 2012) to a third catalog developed by Sewall Young (WDFW, unpublished data). From this alignment we were able to place our RAD-tags on Young's unpublished linkage map, and to remove paralogous sequences. We ranked each SNP in a RAD-tag by the number of individuals genotyped at that SNP and by the frequency of the most common (i.e., major) allele, with the larger number of individuals genotyped and the lower frequency of the major allele receiving the higher score. We selected the one SNP with the highest ranking to represent the RAD-tag; in the event of a tie, we randomly selected a SNP from among the highest ranking SNPs. Finally, we eliminated all SNPs with a minor allele frequency less than 0.05, producing a total of 8598 from STACKS.

We continued to examine the dataset following STACKS to generate a relatively simple first-attempt at associating SNP genotypes with fate. To this end, we eliminated all samples that had fewer than 80% of the SNP loci scored (n=13), or were not identified unambiguously as a survivor or mortality (49), reducing the dataset to 226 individuals (Table 1). Next, based on principal component analysis (PCA) we eliminated outlier sets individuals (Figure 2). Finally, in terms of samples, we removed populations with either low overall sample sizes or extreme differences in the number of survivors and mortalities. However, we retained all samples from the 2014 Green and Nisqually river collections, as these fish were involved in a reciprocal translocation experiment testing the relationship between release location and survival while controlling source location. Therefore, our final dataset included only collects from the Skokomish, Green, and Nisqually rivers; 104 individuals (70 mortalities, 34 survivors) out of the original 288 individuals that were RAD sequenced (Table 1). Finally, we increased the minor allele frequency (MAF) threshold removing all SNPs with MAF less than 0.10, producing a final dataset consisting of 104 individuals and 5702 SNP loci.

We used the mixed linear model (MLM) procedure in the program TASSEL (Yu et al. 2006, Bradbury et al. 2007, Zhang et al. 2010) to provide a preliminary test for associations between fate and genotype. Simply, the MLM attempts to solve: phenotype = genotypes + population structure + family structure (kinship) + residual, with genotypes and population structure being fixed effects and kinship and residuals being random effects. Phenotype is fate (survival or mortalities) plus factors (smolt migration year, source location, and release location; see below). We used the program STRUCTURE, with admixture (Pritchard et al. 2000, Falush et al. 2003, Hubisz et al. 2009), to determine population structure of the three source locations using a reduced dataset consisting of 1043 SNP loci (i.e., all loci that were scored in all 104 individuals). The analysis with K = 4 groups provided the highest likelihood. Q-scores for each individual across the four groups (covariates) sum to 100%, which if all four covariates are included in the analysis will create linear dependency among the covariates. Therefore, as recommended in the TASSEL manual, to prevent the linear dependency we removed the Q-scores for the fourth group or covariate. Pairwise kinship between each pair of individuals was calculated in TASSEL, which calculates kinships as a scaled identity-by-state distance. Finally, we implemented six MLMs each with a different phenotype state (fate + factors): (1) fate only (no factors); (2) Fate + smolt migration year (Year); (3) Fate + smolt source location (Source); (4) Fate + smolt release location (Release); (5) Fate + Year + Source; and (6) Fate + Year + Release. We defined a significance association between a SNP locus and fate visually using quantile-quantile (QQ) plots (locus deviation off of straight line; Figure 3), and as probability ≤ 0.05, adjusted for false discovery rate (FDR; Hochberg and Benjamini 1990, Benjamini and Hochberg 1995). Fate is a categorical phenotype; since TASSEL assumes that the phenotype is a quantitative trait the probability associated for each SNP will be biased low (i.e., showing greater significance).

There was only one SNP locus that was significant or nearly significant in all MLMs: 39529_18 (Figure 3, Table 2). Three other loci were significant for the Fate + Year + Release MLM only: 55970_7, 12301_21, and 51226_71 (Figure 3, Table 2). We used the Basic Local Alignment Tool (BLAST) at NIH's National Center for Biotechnology Information (NCBI) website to match the RAD-tag from each of these four loci to sequences of known identity. The quality of match between the RAD-tag and sequences in the NCBI database is determined by the match's expected-value (E-value); the lower the E-value the higher the confidence in the match. We did not consider matches with E-value greater than 1×10^{-5} (1e-5). The RAD-tags are 80 basepair (bp) long, which is relatively short and resulted in multiple matches for each of the four SNP loci (Table 2). The number of qualifying matches ranged from zero (55970_7) to 14 (51226_71). Across all four loci, the match with the lowest E-value (6e-24) was between 39529_18 and sequences linked to Hox gene clusters in Atlantic salmon. Hox genes control morphogenesis along the anterior-posterior axis, and can be involved with limb (fin) development (Schneider et al. 2011, Pascual-Anaya et al. 2013, Schneider and Shubin 2013, Freitas et al. 2014). Although the match between 39529_18 and the Hox gene cluster was nearly perfect, it occurred in a non-coding part of the Hox gene cluster. 51226_71 matched with 14 different sequences with E-values greater than 1e-5, 13 matching Atlantic salmon sequences, and one matching a rainbow trout/steelhead sequence. The match with rainbow trout/steelhead had the second lowest E-value (2e-13) for this RAD-tag and involved an immunological gene. The match with the lowest E-value (6e-14) was to a sequence linked to Hox gene clusters in Atlantic salmon. In total nearly half of the matches for 51226_71 involved immunological genes (Table 2). As with the 39529_18 matches, none of the matches with 51226_71 occurred in a coding part of the gene. The two matches with 55970_7 had E-values greater than 1e-5 and therefore these sequences did not qualify as significant matches. However, both matches were to Atlantic salmon genes, one involving an immunological gene, and the other a regulatory gene. Finally, none of the five sequences that matched to 12301-21 involved salmonid sequences, and were genomic sequences of unknown function.

This dataset lacked power to provide a definitive association between smolt genotypes 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; (3) fate is a categorical phenotype while the model was built for quantitative data; and (4) the RAD-tag sequences were too short to match more specifically with known sequences in the NCBI database. Nevertheless, there are at least two findings from our analysis that are worth pursuing with additional analyses. First, the HOX gene match with 39529_18 and 51226_71 loci suggests that there may be a developmental difference between survivors and mortalities in morphological features that may be involved with swimming performance (axial and fin development). Second, matches involving immunological genes suggest that there may be a difference between survivors and mortalities in how individual fish respond to pathogens or parasites.

Table 1. Number of samples from each source location and collection year that were RAD sequenced (Complete), had unambiguous fate (mortality or survivor) and sufficient number of loci genotyped (Intermediate), and were included in the final analysis (Final; see text).

Source Location Collection Year	Complete			Intermediate		Final	
	Mortality	Survivor	Ambiguous	Mortality	Survivor	Mortality	Survivor
Big Beef Creek							
2006	8	17	3	8	17	0	0
2007	7	6	0	7	6	0	0
2008	5	3	2	5	2	0	0
2009	3	1	2	2	1	0	0
2010	7	2	3	5	2	0	0
Dewatto							
2006	2	2	0	2	2	0	0
2007	11	2	1	10	2	0	0
Duckabush							
2009	10	3	5	10	3	0	0
Hamma Hamma							
2006	6	6	0	6	6	0	0
2007	4	4	0	4	4	0	0
Skokomish							
2006	6	6	0	5	6	5	6
2007	6	5	0	5	5	5	5
2008	15	5	10	14	5	8	4
2009	6	4	2	6	3	4	3
2010	16	0	0	15	0	7	0
Green							
2008	7	3	4	7	2	7	2
2014	22	9	10	20	9	20	9
Nisqually							
2014	15	5	7	15	5	14	5
TOTAL	156	83	49	146	80	70	34

Table 2. Top: Probabilities for the null hypothesis of no association between SNP locus and phenotype (fate + factor) for the four loci significant at least at one MLM. Probabilities in bold typeface were significant at alpha = 0.05 adjusted for false discovery rate (FDR). Bottom: Total number of sequences in the NCBI database that matched the 80 bp RAD-tag for each locus at E-values ≤ 1e-5 (N), and of those sequences the number whose function can be classified as morphogenesis, immunological, or other.

Phenotype	Locus			
(MLM)	39529_18	55970_7	12301_21	51226_71
Fate	1.54E-05	3.49E-04	5.07E-01	9.14E-02
Fate + Year	**2.92E-06**	2.16E-03	3.96E-01	1.40E-01
Fate + Source	1.42E-05	2.80E-04	4.74E-01	1.51E-01
Fate + Release	1.46E-05	2.63E-04	5.00E-01	1.40E-01
Fate + Year + Source	**3.97E-06**	2.53E-03	3.74E-01	1.21E-01
Fate + Year + Release	**4.18E-06**	**3.62E-158**	**6.63E-117**	**9.44E-83**
N	2	0	5	14
Morphogenesis	1	0	0	1
Immunological	0	0	0	6
Other	1	0	5	7

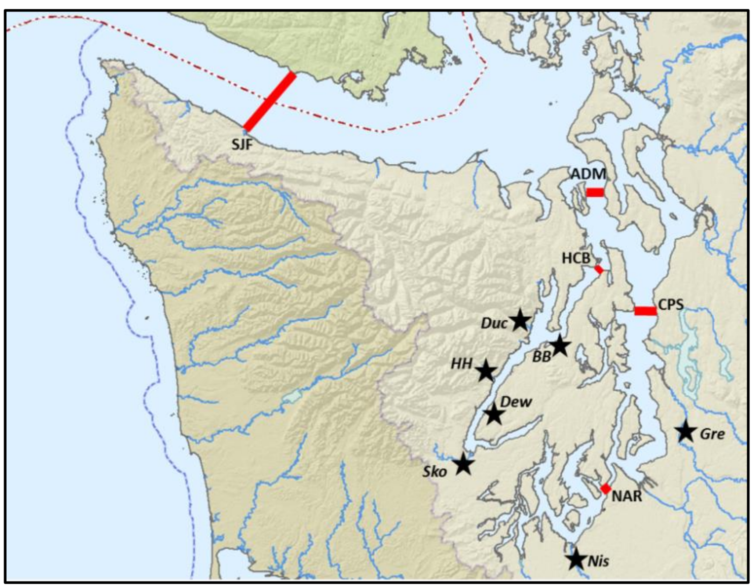


Figure 1. Location of the acoustic receivers (red lines), and general source location of the samples (black stars). Abbreviations: Receivers – Strait of Juan de Fuca (SJF), Admiralty Inlet (ADM), Hood Canal Bridge (HCB), Central Puget Sound (CPS), Tacoma Narrows (NAR); Source locations –Big Beef Cr (BB), Dewatto R (Dew), Duckabush R (Duc), Hamma Hamma R (HH), Skokomish R (Sko), Green R (Gre), and Nisqually R (Nis).

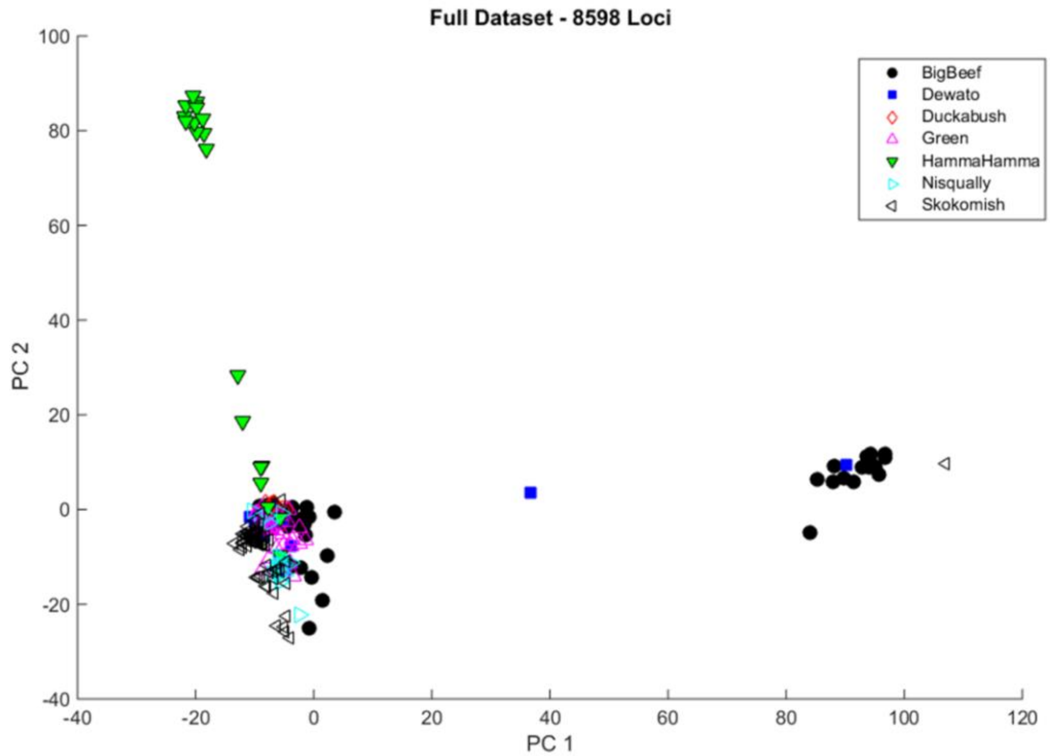


Figure 2. Principal component analysis of all samples using the full 8598 SNP locus data set. Outlier sets of individuals are those that differ from the core set at the lower left of the plot: Big Beef, Dewato, and a single individual from Skokomish along the PC1 axis, and Hamma Hamma along the PC2 axis.

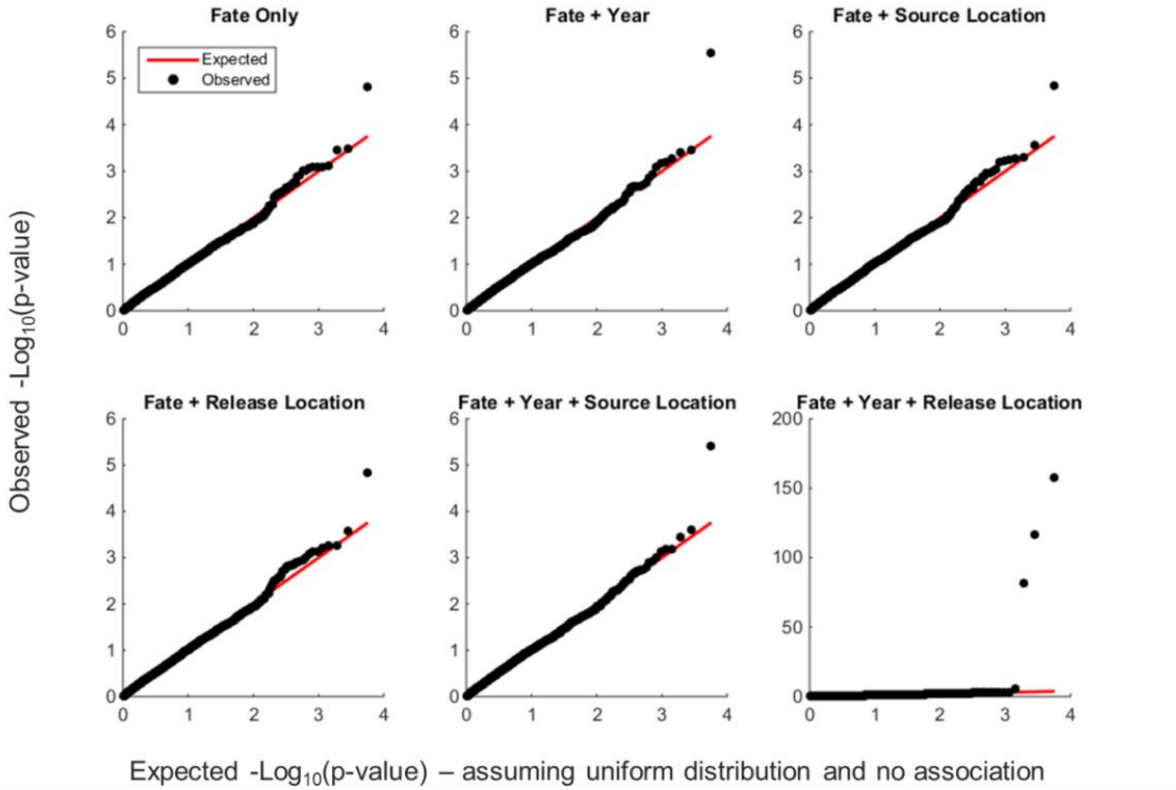


Figure 3. Log quantile-quantile (QQ) probability plots for each of the six MLM analyses. Each filled circle represents a SNP locus (5702 loci in each plot). The red line represents the expected distribution, or null hypothesis of no association between the SNP and fate plus factors. SNPs that appear at a distance from the line indicate a significant association between that SNP and fate plus factors. The one SNP that appears significant in all plots is 39529_18. In the lower right plot, the significant SNPs, from right to left, are 55970_7, 12301_21, 51226_71, and 39529_18. Note differences in scale of y-axis between the plot in the lower right and all other plots

Appendix B: Logic Model Crosswalk with 2013-2015 Research Findings

Freshwater (F) & Marine (M) derived - Poor fish condition and/or altered behavior (ranked)

1. Disease (F/M) – Nisqually & Green (also, Skok & Puyallup?)
2. Outmigrant timing (F)
3. Foraging/Starvation (M) [foraging induced predation maybe. Starvation not likely]
4. Poor water quality/toxics (F/M) – Nisqually
5. Genetic fitness (F) [hatchery introgression not likely. Other driver possible]
6. Outmigrant size/growth (F/M) [not likely]
7. HABs (M) [not likely]
8. Habitat modifications (M) [not likely]

Predator-prey interactions and environmental drivers

1. Predation has increased.
2. Buffer prey decreased
3. Pulse abundance of juvenile salmon/steelhead attracts predators
4. Increased water clarity
5. Low juvenile steelhead abundance

Predation **IS** proximate/ direct cause of mortality

Predation **IS NOT** proximate/ direct cause of mortality

Steelhead dying in Puget Sound

The ultimate source of mortality in Central and South Puget Sound is likely marine derived and not associated with freshwater habitat or hatchery influence. However, causes derived in the lower river, or fish condition effects consistent among steelhead populations, cannot be ruled out. – reciprocal transplant

Evidence/Findings:

- *Nanophyetus salmincola*, with new infections occurring in the lower river, may kill outmigrating steelhead or make these juvenile steelhead more vulnerable to predation, contributing to lower early marine survival rates of steelhead populations in Central and South Puget Sound.
- PBDE's, a contaminant, may affect the health of steelhead leaving the Nisqually River; however, its impact may depend upon the rate it can affect steelhead in the lower river. The other contaminants analyzed and for the Nisqually, Green, and Skagit were less of a concern; however PCBs increased above adverse effects thresholds in samples taken from steelhead collected offshore in Puget Sound.
- Smolts in some populations with particular genetic fingerprints may be compromised by their morphology (fin development) or immunological responses, making them sick or more vulnerable to predation. However, the power of these findings is currently limited. (Nisqually, Green and Skokomish steelhead studied)
- Juvenile steelhead migrating in April and late May survive at higher rates than steelhead migrating in early-mid May. While not yet investigated, this may be associated with factors such as changes in predator-prey dynamics or N. salmincola shedding events/disease outbreaks.
- A steelhead foraging-predation rate relationship was not investigated, but starvation is not likely.
- Whole body lipid content was less than 1.5% in the wild steelhead populations that were assessed (Skagit, Green, Nisqually Low lipid levels are not inconsistent with a decline in whole body lipid content toward depletion during the smolt outmigrant life-stage. However, levels below 1% (in some steelhead) may be cause for concern as 1% has been documented as a threshold for the onset of high over-winter mortality in rainbow trout.
- Juvenile steelhead size at outmigration and steelhead outmigrant abundance are not correlated with survival among years. Size at outmigration is also not correlated with survival within years.

Evidence/Findings:

- An increase in the abundance of harbor seals correlates with the decline in steelhead.
- Abundance trend data are lacking for a correlative assessment with other potential predators.
- Given the significant increase in abundance/prevalence of harbor porpoise, the potential impact should be investigated.
- Initial evidence of correlations with changes in herring abundance (positive correlation), dissolved oxygen (positive correlations) and abundance of hatchery coho (negative correlation) over the period of the decline in Puget Sound steelhead marine survival suggest these factors may be affecting predator-prey dynamics.

Evidence/Findings:

- Steelhead are dying at rapid rates, most within 10 days (likely excludes starvation, & possibly disease, toxics).
- Mortality not highly variable among years (likely excludes HABs, etc)
- The list of most likely, potential bird and marine mammal predators of outmigrating juvenile steelhead includes harbor seals, harbor porpoises, double-crested cormorants, Caspian terns, and Brandt's cormorants.
- Indirect evidence suggests harbor seals are a source of proximate mortality in South and Central Puget Sound.
- Nano-saltwater challenge did not result in direct mortality; however, nano infection was not new.
- Of those contaminants investigated (Total PCBs, Σ_{11}PBDEs, Σ_6 DDTs, HCB, Σ_8Chlordanes, Σ_3HCHs, Σ_{37} PAHs, and estrogenic chemicals) the levels are not high enough to suggest direct mortality.

Evidence/Findings:

- Puget Sound steelhead population abundance and marine survival has declined and remain lower than other nearby regions.
- Puget Sound steelhead early marine survival rates are low, with the highest instantaneous mortality rates in South and Central Puget Sound, and the north end of Hood Canal through Admiralty Inlet.
- Typically, the farther steelhead must swim through Puget Sound, the greater the mortality (death by distance traveled).