Research Work Plan:
Marine Survival of Puget Sound Steelhead

A component of the

Salish Sea Marine Survival Project

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Prepared by the Steelhead Marine Survival Workgroup and contributing experts (see reverse)

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Executive Summary

Puget Sound steelhead numbers are declining dramatically

Wild steelhead – the official Washington state fish – face possible extinction in Puget Sound. Listed as threatened under the Endangered Species Act in 2007, Puget Sound steelhead populations are now less than 10% of their historic size. A significant decline in abundance has occurred since the mid 1980s, and data suggest that juvenile steelhead mortality occurring in the Puget Sound marine environment constitutes a major, if not the predominant, factor in that decline. Millions of dollars have been spent over the past decade 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.

We are implementing critical steelhead research to determine the causes

The Puget Sound Partnership and Washington Department of Fish and Wildlife are coordinating a comprehensive research effort to determine where and why juvenile steelhead are dying in the Puget Sound marine environment. This is a collaborative effort involving federal agencies, Puget Sound Treaty Tribes and the non-governmental organization, Long Live the Kings. The following research work plan consists of eleven studies:

- Five studies use existing data to evaluate patterns and trends in steelhead marine survival and behavior compared to a range of factors that may be contributing to their mortality.
- Four field studies will be conducted to identify the locations, rate and timing of mortality and evaluate disease, toxic contaminants, genetics, and predator-prey interactions to reveal the direct and underlying causes of steelhead mortality in Puget Sound.
- A genetics study will be performed to determine whether there are inherent differences between steelhead that die or survive in Puget Sound, and ecosystem modeling will be used to look at the combined effects of the multiple factors that may be contributing to mortality.

This $1.6 million dollar effort is funded by a $788,000 State appropriation from the Aquatic Lands Enhancement Account (ALEA) in the Puget Sound Partnership budget and an equal match of in-kind and external contributions. This effort is also a component of the Salish Sea Marine Survival Project, a joint US-Canada effort to determine the causes of weak juvenile salmon and steelhead survival in the Puget Sound and Strait of Georgia marine environment.

Recovering the steelhead fishery pays economic dividends

Steelhead fisheries provide major economic benefits, especially for smaller, rural communities around Puget Sound that depend heavily on recreation and tourism spending. The fish support a broad community of sport, commercial and tribal fishers, many of whom have fished in the Sound most of their lives. In 2001, the steelhead fishery in Puget Sound and the Strait of Juan de Fuca generated more than $26 million in annual economic activity. Recovering steelhead and re-establishing steelhead fisheries to levels experienced in the 1970s and 1980s would more than double that amount.

Overview

Wild steelhead – the official Washington state fish – face possible extinction in Puget Sound. Listed as threatened under the Endangered Species Act in 2007, Puget Sound steelhead populations are now less than 10% of their historic size. A significant decline in abundance has occurred since the mid 1980s, and data suggest that juvenile steelhead mortality occurring in the Puget Sound marine environment constitutes a major, if not the predominant, factor in that decline.[1] Millions of dollars have been spent over the past decade 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.

Determining why steelhead are dying in Puget Sound is so important that, in 2012, the Washington Department of Fish and Wildlife (WDFW) identified it as one of the highest priority activities for Puget Sound steelhead recovery[2]. The Puget Sound Salmon Recovery Council, via the Puget Sound Partnership, subsequently funded Long Live the Kings to facilitate steelhead marine survival research action planning, and NOAA Fisheries has requested that the research plan be considered in the Puget Sound steelhead recovery planning efforts now underway. This effort also directly addresses priority actions identified in the 2011-2013 Puget Sound Partnership Science Plan (p. ii, Table ES-1, 9th bullet) and in the Puget Sound Partnership 2012/2013 Action Agenda (p. 93, A6. Protect and Recover Salmon, Tribal Habitat Priorities table, item 6g).

WDFW worked with the Puget Sound Partnership (Partnership), Long Live the Kings, NOAA fisheries, and representatives of the Puget Sound treaty tribes to complete the following comprehensive research work plan to work toward ***determining the causes of and identifying the solutions to juvenile steelhead early marine mortality in Puget Sound***. This effort will primarily be funded by a one-time state appropriation of $788,000 in the Puget Sound Partnership budget and ~100% matching contributions from the project collaborators.

This research plan is a component of the **Salish Sea Marine Survival Project**, a broad US-Canada research initiative coordinated by Long Live the Kings and the Pacific Salmon Foundation to determine the causes of weak survival juvenile salmon and steelhead survival in the Puget Sound and the Strait of Georgia marine

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[1] This doesn't downplay the importance of the freshwater life-stages of Puget Sound steelhead relative to overall survival. To fully support recovery actions, a comprehensive analysis of the effects of factors affecting survival in the freshwater as well as marine factors is needed.

[2] WDFW 2012.

environment. On the US side, a multi-disciplinary scientific Technical Team is leading the research development for Puget Sound.

The Steelhead Marine Survival Workgroup (Workgroup) who developed this work plan and will implement the research consists of WDFW staff, members of the Salish Sea Marine Survival Project's Technical Team, and other biologists who represent relevant disciplines or who have studied steelhead in the marine environment. The core Workgroup participants and the numerous other scientists who contributed to the development of this plan are listed as contributing authors at the beginning of this document. The research planning effort has been coordinated by Long Live the Kings.

The early marine survival research proposed for steelhead is aligned with research activities being developed for the other salmon species assessed via the Salish Sea Marine Survival Project[3]. The steelhead early marine survival research plan has been reviewed by the Marine Survival Project's Coordinating Committee, the Salish Sea Technical Team, members of the Puget Sound Recovery Implementation Technical Team, and other interested parties. Reviewer comments and associated Workgroup responses are in Appendix 4: Responses to reviewer comments.

As there is no clear smoking gun for the causes of mortality, a multi-disciplinary, ecosystem-based research approach is being applied. Historic analyses of existing data, field studies and experiments to collect and analyze new data, and modeling to pull it all together will be used to examine current assumptions about where and why steelhead are dying. The research process is stepwise, with each research activity informing next steps. Some of the research actions described in this plan are already underway, and their initial results have guided the development of this work plan.

The steelhead research framework addresses the following questions:

1. 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?
2. What is the direct/proximate cause of mortality in Puget Sound?[4]
3. What is leading to this mortality? What are the root/underlying causes? Are they freshwater and/or marine derived?

The steelhead Workgroup reviewed, discussed and categorized the existing evidence, their assumptions and their recommended research needs within this framework of questions. The assumptions are summarized in the diagram below (**Figure 1**). The evidence supporting each assumption and

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[3] For more information about the Salish Sea Marine Survival Project, go to: www.tinyurl.com/marinesurvival.

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

recommended research needs are described in the next section. Study proposals; a description of the project management, communications and outreach approach; the proposed work schedule; and the budget follow.

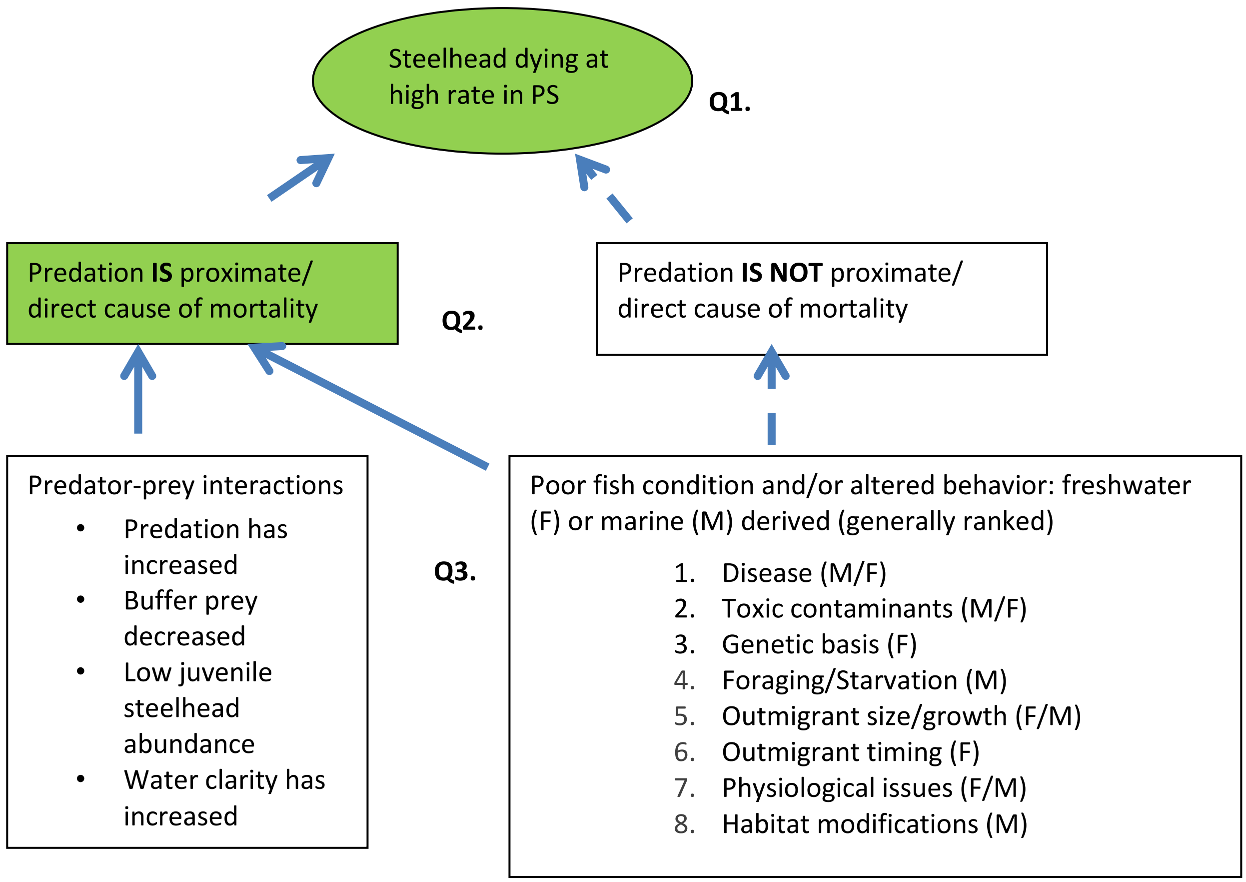

Figure 1. Puget Sound steelhead marine survival evaluation: The green color indicates where the group generally agrees with the evidence. The factors that may be affecting fish condition or behavior are also ranked based upon existing evidence.

Ultimately, an additional step beyond this research is to determine whether the factors affecting survival are fundamentally the result of local human-induced pressures (e.g., habitat change, toxics, species management) or larger shifts (e.g., from climate and ocean variation, temperature and OA) differentially affecting an inland sea (when compared to the coast). For example, larger-scale habitat changes, such as temperature regime shifts associated with climate change or ocean cycles, could fundamentally be affecting the prevalence of disease, presence and abundance of buffer prey, changes to turbidity, and/or changes to marine predator migration and residence patterns.

Review of Evidence, Assumptions, and Recommended Research Needs

1) What is the survival history of Puget Sound steelhead and where, when, and at what rate is mortality occurring now?

Adult abundance, smolt-to-adult survival rates, and telemetry-based early marine survival estimates have been used to evaluate the survival of Puget Sound steelhead relative to other regions and determine where, when, and at what rate mortality is occurring in Puget Sound.

1.1 Trends in Adult Abundance and Smolt-to-Adult Survival Rates[5]

Preliminary analyses of wild steelhead adult abundance trends and wild and hatchery smolt-to-adult survival rates ('SARs', which are primarily a reflection of survival in the marine environment) suggest that steelhead populations along the Pacific Coast, from British Columbia through Oregon, shared a pattern of declining abundance from the mid 1980s through the mid 90s.[6] The shared pattern suggests common, Pacific region-level factors such as climate and ocean conditions were driving survival. While steelhead along the Washington coast and from the Columbia River rebounded in the 2000s, adult abundance and hatchery SARs for Puget Sound continued to decline. Since then, SARs for Puget Sound populations have generally remained below those of other regions. Furthermore, the magnitude of the difference in SARs when comparing Puget Sound to Washington coast populations has increased. Unless Puget Sound steelhead are experiencing greater mortality in the open ocean than Washington coast and Columbia River populations, this information suggests that mortality has increased in Puget Sound. See **Figures 2-4** at the end of this document.

Within Puget Sound, Snow Creek (draining into the Strait of Juan de Fuca) wild steelhead abundance and SARs have on the whole declined since the late 1970's. Their SARs are currently higher than Nisqually River and Big Beef Creek steelhead (in Southern Puget Sound and Hood Canal, respectively), two of the other Puget Sound wild steelhead populations with smolt-to-adult (marine) survival data (**Figure 5)**. Puget Sound wild steelhead abundance (**Figure 2**) and hatchery steelhead SARs (**Figure 6**) also suggest that populations originating in South Sound and Hood Canal have been

[5] The SAR data in this section represents initial findings from a comprehensive assessment currently underway (Kendall et al 2013, WDFW unpublished data). WDFW staff will complete this assessment in the fall of 2013, as described in the Research Components section, below.

[6] North Pacific-wide wild steelhead abundance trend data can be found at http://www.psmfc.org/steelhead/past-2012.html. The wild steelhead abundance data presented in this report compares Puget Sound to the Washington Coast, and the hatchery SAR data compares Puget Sound to the Washington Coast and Lower Columbia River. Some Strait of Georgia abundance and SAR data is also presented for comparison.

impacted more greatly over time and that higher mortality continues to occur for south/south central Sound and Hood Canal populations compared to north Puget Sound and the Strait of Juan de Fuca.

There are some indications that low steelhead marine survival may be a Salish Sea-wide phenomenon. In the Strait of Georgia, steelhead populations in the Keogh River, near the north end of the basin, and the Chilcotin and Thompson rivers, Fraser River tributaries in the south end of the basin, share the same pattern of decline in abundance as Puget Sound stocks, and Keogh River marine survival rates (the only Strait of Georgia river with such data) have declined significantly. However, small steelhead (hatchery and wild) populations in the Englishman and Chekamus rivers, which enter the lower Strait of Georgia and the Coquihalia River, a tributary of the Fraser River, appear to be relatively stable or increasing in abundance. **See Figures 7-10**.

The potential causes of lower Puget Sound population marine survival rates compared to other regions could include:

Cause of lower SAR	**Data to support**
Low survival of smolts through Puget Sound	**Telemetry data suggest 19.7% of wild steelhead survive the trek through Puget Sound (see section 1.2).** This may be a minimum survival estimate. No data on coastal early marine survival (first two weeks of ocean residence)
Low survival of adults as they return to Puget Sound and before they enter the rivers.	No hard data on this, but possible. Nisqually Tribe staff have observed heavy pinniped predation on adult steelhead.[7]
Low survival in the ocean because they experience different ocean environments than other stocks (i.e., they migrate to different places)	No evidence that Puget Sound steelhead migrate to different regions than, for example, coastal stocks, but this has not been examined. Variation in the timing of Puget Sound steelhead entering the Pacific Ocean has also not been thoroughly examined.
Combined effects of two or more of the above	

[7] Pers. comm. C. Ellings, Nisqually Tribe, 2013.

1.2 Early Marine Survival and Locations of Mortality (Acoustic Telemetry Data)[8]

From 2006-2009, nearly 1,400 steelhead smolts from 9 watersheds within Puget Sound were tracked from river mouth to the Pacific Ocean using acoustic telemetry to: (1) estimate survival through Puget Sound ('early marine survival'), (2) identify common areas of abnormally high mortality along the migration route, and (3) identify factors that may influence survival. Cormac-Jolly-Seber mark-recapture models were used to jointly estimate survival and detection rate at telemetry arrays.

Estimated survival probabilities from river mouths to near the Pacific Ocean ranged from 2.7% (White River hatchery smolts in 2009) to 44.8% (Skokomish River wild smolts in 2006), and averaged 16.8% for all populations. Factors influencing survival included population, migration year, and rearing type (i.e., hatchery or wild). Geographic region, body length, and tag size (i.e., 7 mm or 9 mm) showed lesser effects. Hatchery populations tended to survive at lower rates relative to their founder wild populations (average wild survival: 19.7%; average hatchery survival: 11.7%). Survival probabilities were broken up by migration segment, and distinct patterns of mortality were observed among populations by sub basin. Variation in survival among migration segments indicated Central Puget Sound and Admiralty Inlet were potential areas of heightened mortality ('mortality hotspots'). Steelhead smolts spent very little time migrating though Puget Sound. River mouth to Pacific Ocean travel times ranged from an average of 6.2 days (Green River smolts) to 17.4 days (Skokomish River smolts), suggesting that smolts were traveling at or near maximum sustainable swimming speeds for some or all of their migration to the Pacific Ocean. The rapid travel rates narrow the list of potential mechanisms causing mortality in Puget Sound. This study, to be submitted for publication in 2014, addresses a major gap in steelhead marine life history knowledge and can help to inform future Puget Sound steelhead recovery planning efforts. **See Figure 11** for a description of the Puget Sound early marine mortality rates by population and migration segment, and **Figure 12** for modeled depiction of daily mortality that occurs in Puget Sound over a three week time period relative to the daily mortality rates that would need to be sustained in the Pacific Ocean to achieve an overall SAR of 3.0%.

Wild and hatchery steelhead smolts tracked from rivers feeding into the Strait of Georgia survived at rates similar to those estimated in Hood Canal and displayed similar migration behavior (Melnychuk et al. 2007, Welch et al. 2011). The low early marine survival rates common throughout the Salish Sea suggest a widespread, not localized, problem.

[8] This section represents the initial results of the work of NOAA Fisheries (Moore et al. 2013, NOAA unpublished data) and Moore et al. 2010. NOAA staff will complete this work in the fall of 2013, as described in the Research Components section, below.

1.3 What do the current analyses suggest?

Evidence generally suggests that Puget Sound steelhead marine survival has declined and now is generally lower than other regions outside of the Salish Sea. Additionally, although we cannot exclude the other potential causes of lower Puget Sound population marine survival rates, high rates of early marine mortality likely explain the difference. Furthermore, the SAR and telemetry data, combined, suggest that, for any hypothesis to explain the higher steelhead mortality in Puget Sound, the following conditions should apply:

- The cause(s) of mortality must have become more severe over the past 25-30 years and sustained since the mid 1990s.
- The cause(s) of mortality must be less severe in other regions (e.g., coast, lower Columbia River, etc), at least periodically.
- The cause(s) must impact steelhead within one to two weeks after seawater entry (latent effects are possible but do not explain the telemetry-based survival estimates).
- The cause(s) of mortality must result in higher concentrations of mortality in central Puget Sound and Admiralty Inlet ('mortality hotspots').
- The cause(s) of mortality must result in higher mortality to south/south central Sound and Hood Canal populations than north Puget Sound and the Strait of Juan de Fuca.
- The cause(s) of mortality must have a greater relative effect on hatchery populations compared to wild populations.

1.4 What's next?

Preliminary results of ongoing research activities performed by WDFW and NOAA have been used in the description of what we know to date. This work will continue, and the results will be used to inform the study process.

This regional and population-specific assessment will provide the foundation for correlative analyses with factors potentially affecting survival. Abundance and SAR data will continue to be analyzed in order to evaluate hypotheses about local vs. regional/ocean drivers, assess variation within Puget Sound and the Strait of Georgia[9] to better support or refute the current assumptions about the likely survival drivers, provide additional reference regarding whether and where mortality is concentrated, and account for individual or population characteristics that may differentially affect survival. Additional telemetry work will provide a more refined picture of where (to help isolate areas of higher mortality), when, and at what rate mortality is occurring in Puget Sound. Finally, Puget Sound research will be aligned with work in the Strait of Georgia to the greatest extent practicable given the similarities in trends among several of the Salish Sea populations.

[9] Including why some small Georgia basin populations may not be adhering to the trends seen elsewhere in the Salish Sea.

A complete description of the research activities associated with question 1 are described in the research components section on page 27.

2) What is the direct/proximate cause of mortality in Puget Sound?

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

2.1 Review

The Workgroup generally agrees that predation is likely the direct cause of mortality in Puget Sound. This is supported by the following evidence:

- Acoustic telemetry data indicate that steelhead are migrating quickly and mortality is occurring at a rapid rate within Puget Sound, with most steelhead migrating through the system or dying within one to two weeks. This rapid rate of migration and mortality suggest that starvation is not the direct cause of mortality, given that, in controlled environments, steelhead can go for longer than two weeks without food (pers. comm. B. Berejikian, NOAA 2013). Direct mortality resulting from disease is also unlikely within this time period.
- Acoustic telemetry data indicate hotspots within Puget Sound that overlap with areas of higher predator concentration. (see **Figure 11,**
- **Figure** 18**, and Figure 17**)
- Acoustic telemetry and SAR data indicate that the mortality is not highly variable among years, suggesting that mortality is not caused by factors with greater variability in the environment such as harmful algae blooms. However, additional retrospective work is recommended to support or refute this hypothesis.
- Steelhead tend to migrate near the surface, making them susceptible to avian and some marine mammal predation.[10]

2.2 What's next?

The Workgroup concluded that simply determining whether predation is the direct cause of mortality would leave many unanswered questions because it does not answer why the mortality is

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[10] Juvenile steelhead have been found to migrate in the top 1-3 meters of the water column in the Columbia River plume (pers. comm. K. Fresh, NOAA 2013). Beeman and Maule(2006) also found juvenile steelhead to migrate in the top 2 meters in freshwater as they migrate out of the Columbia River.

occurring and has apparently increased since the 1980s. Through the research activities proposed below, predation will be evaluated in a broader context.

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

Two categories were established to describe what may be leading to juvenile steelhead mortality in Puget Sound (assumed to be predation): a) changes to predator-prey interactions in the marine environment and b) poor fish condition and/or altered behavior as a freshwater and/or marine factors. Factors and their attributes are qualitatively assessed for their potential contribution to juvenile steelhead mortality (green = high probability, yellow = moderate to low probability) based on the conditions described in section 1.3 and what we know about the size and behavior of outmigrating steelhead. The assessment is summarized in **Figure 1**, above.

3.1 Are the root causes of mortality freshwater or marine derived?

At a high level, the SAR and telemetry data combined with what we know about the freshwater and marine environments provides general evidence regarding the origin of the underlying causes of mortality. SAR declines, low SARs compared to other regions and, where data are available, high early mortality rates are generally shared among populations within Puget Sound and to some extent the Strait of Georgia. This suggests that the cause(s) of mortality are shared. Therefore:

1. If the cause(s) of mortality are shared and freshwater derived, they would have to occur at similar levels across numerous, disparate watersheds that range from having very intact habitat and little hatchery influence to watersheds greatly influenced by habitat modifications and hatcheries.

2. If the factor(s) affecting steelhead survival are shared and marine derived, they would need to either: a) be broadly distributed or b) result in concentrated mortality in locations where multiple populations pass through.

Parent spawner-to-smolt progeny survival rates in Snow Creek between 1981 and 2009 show no signs of a worsening situation in the freshwater environment (**Figure 13**). While this doesn't discount the possibility that smolt outmigrant condition is negatively affected by the freshwater environment, one could expect a change in freshwater survival rates if fish condition were deteriorating. However, Snow Creek enters the Strait of Juan de Fuca and is not representative of inner Puget Sound and Hood Canal populations, where the decline in SARs has been most apparent.

3.2 Changes to predator-prey interactions in the Puget Sound marine environment

The following three factors may be contributing to changes to predator-prey interactions in the Puget Sound marine environment: 1) increase in predator abundance or change in predator distribution, 2) decrease in 'buffer prey' species, and 3) changes in juvenile steelhead abundance.

3.2.1 Increase in predator abundance or change in predator distribution

Predation pressures on steelhead are likely exerted by a variety of mammalian, avian and piscine predators. Potential predators have been preliminarily evaluated based upon the following criteria[11]:

- Does the predator eat juvenile salmon? Does the predator eat juvenile steelhead?
- Does the predator feed near the surface, in the first 1-3m[12], at the depth steelhead typically outmigrate?
- Is there an overlap in size distribution between predator diet and juvenile steelhead? [Wild steelhead smolt size distribution is 125-230mm (mode ~170)[13] (see **Figure 14**), and hatchery steelhead are typically released at 180-210 mm[14]]
- Is the predator reasonably abundant in Puget Sound and Strait in May and June? [Wild steelhead typically outmigrate in mid-April through June (peak in May)[15], and hatchery steelhead are typically released from late April through mid-May[16]}
- Is the predator population increasing? AND/OR has there been a change in distribution?
- Is the predator abundant in the area where juvenile steelhead are apparently dying?

Few, if any juvenile steelhead have been identified in predator diet composition work for Puget Sound. This could be due to the time of year when diet studies have occurred (not aligned with steelhead outmigration) or, more likely, the low abundance of steelhead relative to other available prey. In the Columbia River, juvenile steelhead are primarily consumed by Caspian terns, double-breasted cormorants, and common mergansers (Weis et al. 2008). However, diet observed in one location/time may not be indicative of diet in another location/time. Diet composition of piscivorous marine mammals (Lance et al. 2012) and birds (Pearson et al. in review 2013) vary dramatically seasonally and over small spatial scales. For example, rhinoceros auklet diet in the California Current of Washington varies dramatically from that observed in the Salish Sea only tens of kilometers away (Pearson et al. in review). However, prey size distribution data suggest diet overlap for certain predators. They are listed below. Please note that these are preliminary results: this is a work in progress.

[11] The predator evaluation represents initial findings from a comprehensive literature review currently underway (Pearson et al 2013, WDFW unpublished data). WDFW staff will complete this assessment in the fall of 2013, as described in the Research Components section, below.

[12] See footnote 10.

[13] Pers. comm. B. Berejikian, NOAA, 2013.

[14] Hatchery release date range from WDFW fish plan database. Provided by K. Henderson, WDFW, 2013.

[15] Pers. comm. B. Berejikian, NOAA, 2013..

[16] Hatchery release date range from WDFW fish plan database. Provided by K. Henderson, WDFW, 2013.

Piscine predators have not been thoroughly evaluated; however, most of the potential piscine predators (resident Chinook/coho, dogfish, six-gill or salmon shark, sebastes, lingcod, and other larger gadids) are assumed to be more active predators deeper in the water column than where steelhead outmigrate. A cursory evaluation of piscine predation was performed and is discussed in Appendix X. Responses to Reviewer Comments.

Of the potential ***avian predators***, cormorants, Caspian terns, common mergansers, and loons do consume prey the size of steelhead outmigrants in Puget Sound. Of the four, cormorants are the most abundant during the steelhead outmigration time period. For example, pelagic cormorant ranges from 380-1067 birds from May 15-end of July) (pers. comm. S. Pearson, WDFW, 2013). However, this population size is not large relative to other potential predators. The rhinoceros auklet population is large and has increased significantly in Puget Sound since the 1980s. In 1980, ~17,000 breeding pairs (34,000 total individuals) were nesting on Protection Island, the location of the largest nesting colony of rhinoceros auklet (Grover and Olla 1983). From 2003-2009, an estimated 72, 304 auklet were nesting at Protection Island and 3,092 at Smith Island for a total of 75,396 auklet (Pearson et al. 2013). Rhinoceros auklet population estimates from May 15 through end of July, 2003-2009, in Puget Sound range between 21,742 and 41,719 birds. Rhinocerous auklets forage in Admiralty Inlet, a hotspot for steelhead mortality. However, there is only a small amount of overlap in size distribution between rhinoceros auklet diet and the size of outmigrating steelhead (pers. comm. S. Pearson WDFW 2013). If rhinoceros auklet were a primary predator, one may expect to see size-selective mortality (with fewer, smaller fish surviving) reflected in the Puget Sound telemetry data. Diet of adult rhinoceros auklets also includes salmon (Lance and Thompson 2005) but because salmon prey were only identified to genus in past studies, we don't know if steelhead are consumed. No steelhead were identified in 5,705 individual fish samples (all salmon samples were identified to species genetically) intended for chicks (pers. comm. S. Pearson WDFW 2013).

The ***marine mammals*** most likely contributing to predation are harbor seals and harbor porpoises. Both mammals feed on fish the size of steelhead, have increased in abundance in Puget Sound (and the Strait of Georgia), and are in Puget Sound in large numbers during the steelhead outmigration period.

Harbor Seals – Puget Sound and Hood Canal steelhead smolts must migrate past dozens of harbor seal haul-out areas en route to the Pacific Ocean, which likely presents a higher encounter rate with predators than for coastal populations (see **Figure 17**). The abundance of harbor seals in greater Puget Sound[17] increased significantly between the 1970s and 1999, from 2,000-3,000 to nearly 14,000(see **Figure 15**). Concomitantly, significant increases have also been reported for the Strait of Georgia, with the population increasing from a few thousand in the early 1970s to 40,000 in the mid 2000s (see **Figure 16**) . However, harbor seal populations in both Puget Sound and the Strait of Georgia reached maximum abundance around 1999, when they presumably reached carry capacity (Jeffries et al. 2003 and Trites 2012).

[17] Includes Puget Sound, Hood Canal, San Juan Islands, East Bays and the Strait of Juan de Fuca as described in Jeffries et al. 2003.

Harbor seals are opportunistic and feed on a range of prey sizes that encompasses the size range of steelhead smolts. They typically prey on whatever fish species are abundant within 20km of their haul-out site (Trites 2012). Harbor seals feed on adult salmonids during return seasons, and small schooling fish and gadid species throughout the year (Lance and Jefferies 2007). Steelhead are not schooling fish. Also, high mortality rates have not been observed at narrow estuarine mouths where fish are consolidated, which is what one would predict if steelhead are being eaten predominantly by seals (pers. comm. S. Jefferies, WDFW 2012). Furthermore, steelhead were not identified as prey in a fairly extensive year-round analysis of harbor seal diet around the San Juan Archipelago (Lance et al. 2012, Bromaghin et al. 2013). However, steelhead presence in diets may have been overlooked due to the lack of precision in the diet composition analysis and because the relative number of steelhead available as prey compared to other prey types is inherently small. Additionally, steelhead would not have to represent a large portion of the harbor seal diet for a significant impact on steelhead population mortality to occur. As an example of potential predation impact for Hood Canal, a population of 1,000 harbor seals (c.f. Jeffries et al. 2003) with a daily mean diet composition of 0.5% steelhead smolts and consumption rates of 2 kg/individual/day (Howard et al. 2009) could consume half of the estimated 40,000 smolts from Hood Canal over the course of the approximate two-month steelhead outmigration period.

Are Puget Sound Steelhead in a predator pit?

The concept of predator pits assumes that there is a low density equilibrium separated from a high density, relatively stable equilibrium (abundance). Prey populations are in the "predator pit" when their yearly losses to predation are greater than their yearly population gains (Seip 1995). Prey populations in the predator pit will decline to the lower equilibrium. The key requirement for two different equilibria is a functional response on the part of the predators such that the per capita risk of being taken by a predator actually increases with increasing prey density in the neighborhood of the lower equilibrium but decreases in the neighborhood of the higher equilibrium… In this context, equilibrium does not mean constant predator and prey densities, but that densities tend to return to the vicinity of the equilibrium if they are caused to deviate substantially from it (see **Figure 19**).

(Excerpt from the Committee on Management of Wolf and Bear Populations in Alaska, National Research Council 1997).

Harbor Porpoises – Like harbor seals, the abundance of harbor porpoises has increased significantly in Puget Sound in recent years (Figure 18). Based on surveys conducted in 1990-91, the estimated harbor porpoise abundance for Inland Washington was 3, 352 (CV = 0.270), although this may have been an overestimate (Osmek et al. 1996). Population estimates from May 15 through the end of July (during the juvenile steelhead outmigration period), 2003-2009 in the Salish Sea, range 2,455-5,608 porpoises (S. Pearson WDFW, 2013, unpublished data). The most recent census of harbor porpoises in Puget Sound in 2003 estimated their number at

10,680.**[18]** Figure 18 illustrates the increase in number of harbor porpoise sightings from 1993-2011. The figure also illustrates the high concentration of harbor porpoises in Admiralty Inlet, an area where outmigrating steelhead experience a higher levels of mortality (see **Figure 11**). Harbor porpoise prefer fish 80-250 mm in length (Hall 1994, Toperoff 2002). Harbor porpoise also tend to reside high in the water column[19], consistent with where steelhead outmigrate. Their diet is predominantly herring, eulachon and sand lance, aligning well in size with steelhead smolts.

Dall's porpoise - Dall's porpoise have similar food habits to Harbor porpoise; however, they may forage deeper in the water column (Walker et al. 1998). Little is known about the Dall's porpoise population size in Puget Sound, but it is assumed to be small relative to harbor porpoise.

Steller sea lions - Steller sea lion populations have also increased in abundance; however, they typically leave Puget Sound for their rookeries in June and therefore are not present over the entire steelhead outmigration period (pers. comm. S. Pearson WDFW 2013). Their preferred prey appear to be small or medium-sized schooling fishes (Lance and Jeffries 2007) and their prey size varies from several cm to over 60 cm (NMFS 2008b). Walleye pollock and Atka mackerel have been identified as dominant prey followed by Pacific salmon and Pacific cod; predominately late-stage juvenile and adult sized fish (>250 mm; Sinclair and Zeppelin 2002).

3.2.2 Decrease in 'buffer prey' species

Buffer prey[20] species likely include various species of forage fish, including herring and resident Chinook and coho salmon in the same size range as outmigrating steelhead. Herring and pacific sand lance (at least historically) comprise(d) the major part of the neritic forage fish assemblage in Puget Sound (Hiss 1986) and likely represent a majority of the total fish biomass available to predators during the steelhead outmigration period.

The overall abundance of herring in Puget Sound has declined significantly since the 1970s. Cumulatively, south and central Puget Sound herring stocks have remained relatively stable since the 1970s, while the Cherry Point stock, which historically represented half of the total Puget Sound herring spawning biomass, and the cumulative north Puget Sound (excluding the Cherry Point stock) and Strait of Juan de Fuca regional spawning biomasses are at low levels of abundance (Stick and Lindquist 2009) (see Figure 20). There are both resident and migratory herring populations, and the seasonal movement of migratory populations affects their overall abundance in Puget Sound. Migratory populations spend late spring, summer and fall months feeding on shelf waters off west coast Washington State and Vancouver Island. They return to the Salish Sea in fall months (October-December) forming dense, overwintering concentrations

[18] Based upon the work of Steve Jeffries (WDFW), referenced in the Olympian article, Harbor porpoises making a comeback in South Sound, http://www.theolympian.com/2013/02/27/2440489/harbor-porpoises-making-a-comeback.html#storylink=cpy

[19] http://www.nps.gov/klse/naturescience/whales.htm.

[20] Buffer prey = an alternate prey source for steelhead predators whose abundance may affect the predator burden on steelhead.

(Therriault et al. 2009). The herring spawning season extends from late January through mid-June. Most Washington State herring stocks spawn between mid-January and early April. The exception is the Cherry Point stock in north Puget Sound, which spawns from early April through early June (WDFW 2012). The Cherry Point spawn time is notable because it overlaps with the steelhead outmigration period and both Puget Sound steelhead and Cherry Point herring have declined significantly over similar time periods.

It also worth noting the collapse in age structure of Puget Sound herring. Data from the mid 1980s to 2006 demonstrate that all Puget Sound stocks share a collapse in age structure (Landis and Bryant 2010), with fewer fish of older ages being observed. This may represent a significant decline in larger prey available to the predators listed above, which may compound the reduction of the buffer effect herring may have historically provided for outmigrating steelhead.

Eulachon, which were ESA-listed as threatened in 2010, were also historically abundant in the Strait of Georgia and Strait of Juan de Fuca marine environment during the steelhead outmigration period. Eulachon had a strong presence in the Strait of Georgia (Fraser stock), but that population has collapsed. Eulachon did historically spawn in the Elwha River in the Strait of Juan de Fuca; however, there is no record of eulachon spawning in Puget Sound.[21]

Other potential buffer prey species that have also declined in Puget Sound consistent with the decline of steelhead include resident Chinook (RMIS 2012), coastal cutthroat, Pacific hake (NMFS 2009), Pacific cod (NMFS 2011), rockfish (NMFS 2008a) and walleye Pollock (EoPS 2013).

3.2.3 The role of juvenile steelhead abundance

Both hatchery steelhead production and wild smolt outmigration abundance have varied since the late 1980's when steelhead marine survival rates began to decline. High numbers of outmigrating fish over a short period can overwhelm predators' capacity to attack, handle and consume prey (Ims 1990). Alternatively, low abundances of prey may not attract the attention of predators and reduce the potential for predation (Bakun 2006).[22] Both situations can result in higher survival for the group of outmigrants. These effects may occur at the population, sub-region (south PS, central PS, north PS, Hood Canal, SJDF), or Puget Sound region level. An initial analysis of Snow Creek steelhead data indicates a weak negative correlation between juvenile outmigrant abundance and overall marine survival (see **Figure 21**).

3.2.4 Water clarity may directly impact predator-prey dynamics

Water clarity can impact the susceptibility of fish prey to predation. Throughout Puget Sound, water clarity has on average increased since 1998 (see Figure 23). Increased

[21] Information from http://www.seadocsociety.org/taxonomy/term/36 and http://www.gpo.gov/fdsys/pkg/FR-2010-03-18/html/2010-5996.htm.

[22] Conversely, the relationship between juvenile steelhead abundance and marine survival in Puget Sound could be correlated with density-dependent effects such as competition in the freshwater, resulting in decreased fish condition at outmigration, or competition in the marine environment. However, the small relative abundance of steelhead compared other species at similar trophic levels during marine outmigration may suggest otherwise.

water clarity (reduced turbidity) can increase predation by piscivorous fishes by increasing predator-prey encounter rates (Gregory 1993; Gregory and Levings 1998; De Robertis et al. 2003). Increased water clarity can also affect predation by birds, potentially increasing encounter rates of pursuit-diving predators (e.g., cormorants) (Strod et al. 2008 and Hostetter et al. 2012). Alternatively, more turbid water may allow pursuit-diving birds to get closer to their prey (Ainley 1977, Henkel 2006). Foraging success of animals that locate their prey with echolocation (e.g., cetaceans) may be enhanced in turbid water, where prey are less able to detect predators (Henkel and Harvey 2008, Abrahams and Kattenfeld 1997). Alternatively, predation by visual predators (e.g., sea lions) may be reduced by turbid water (Henkel and Harvey 2008). Increased turbidity may also affect predators that use both vision and tactile senses (e.g., harbor seals); however, these predators may be able to compensate for a reduction in one modality through the other (Weiffen et al. 2006). For example, according to Henkel and Harvey (2008), the distribution of harbor seals in Monterey Bay, California was not influenced by water clarity.

3.2.5 What's next?

The evaluation of potential predators will be completed and an initial study of predator encounter rate will be performed to determine whether, and if so, at what rate predation is occurring. The initial results suggest that harbor seals and harbor porpoises are the most likely predators, and the existing acoustic telemetry network provides a mechanism for evaluating them. Of the two, it is more feasible to evaluate harbor seals because harbor porpoises are difficult to capture and tag. The Workgroup will also investigate the potential use of retrievable critter cams mounted on harbor seals or harbor porpoises to detect what they are eating. A PIT tagging/retrieval study was also discussed to evaluate bird predation; however, this was considered of secondary importance since birds are currently assumed to have a nominal impact. Low juvenile steelhead abundance and their limited Puget Sound residence time relative to other prey makes other approaches (predator diet composition and bioenergetics) to evaluating predator-prey interactions difficult.

To the extent practicable, bioenergetics and ecosystem models developed via parallel efforts, as part of the broader Salish Sea Marine Survival Project and/or elsewhere, will be used to expand the results of retrospective and field work and allow for the evaluation of multiple factors (cumulative effects). Buffer prey (i.e., herring) abundance and juvenile steelhead abundance relative to marine survival rates will also be analyzed and the information will be used to evaluate cumulative effects. Similarly, turbidity will be coarsely evaluated as an environmental factor that could influence predation rates, and thusly, survival.

Finally, while marine mammal and bird assessments have periodically overlapped with the steelhead outmigration period, that time frame was not the focus. Also, total abundance in Puget Sound has not been estimated for certain species of interest for some time. The Workgroup discussed the value of performing a marine mammal and predator census during the outmigration period, but determined it was too costly to justify (very difficult to relate predator abundance to steelhead mortality without additional information about the predator-prey relationship since steelhead abundance is low relative to other prey available) and concluded that related information, while not targeted on the steelhead outimigration window, could be gathered from proposed or ongoing studies.

3.3 Poor fish condition and/or altered behavior

While changes to predator-prey interactions likely are contributing to the increased mortality of juvenile steelhead in Puget Sound, the low abundance of steelhead relative to the buffer prey populations and that steelhead are not a schooling species (which is what the identified potential predators typically target) suggest that other factors may be contributing to steelhead mortality. Steelhead may be more susceptible to predation than other fish species because they migrate high in the water column, because they are relatively large at outmigration, or because buffer prey abundances have decreased. Alternatively, the condition or behavior of outmigrating steelhead may have changed and may now be contributing to lower survival. The following paragraph from Hostetter et al. 2012 summarizes the theory of condition-dependent predation:

> The theory that predators disproportionately prey on individuals that are in substandard condition (e.g., weak, sick, stressed, or inexperienced; sensu Temple 1987) is widely accepted and has been well supported in fish predation studies (see review by Mesa et al. 1994). The occurrence and magnitude of condition dependent predation may vary as a function of predator foraging strategy. In theory, predators that chase their prey should be more likely to disproportionately take individuals in poorer condition compared to predators that ambush their prey (Estes and Goddard 1967; Schaller 1968). Studies evaluating predator–prey interactions and the efficacy of predator management, however, rarely consider the influence of prey condition and predator foraging strategies (Mesa et al. 1994). For instance, the success of predator management efforts to increase prey populations would be diminished if the prey would have died from other causes (e.g., disease, competition, or other predators) regardless of the predation event (Errington 1956; Temple 1987). Thus, the degree to which the mortality caused by predation is compensatory is a primary consideration for programs that seek to restore prey populations through predator management.
>
> Hostetter et al. 2012

The following summarizes the factors that may be affecting fish condition and/or behavior. Data to support or refute the role of fish condition and/or altered behavior in early marine mortality are extremely limited. Absent the data, the Workgroup received the perspective of numerous experts to help categorize the likelihood certain factors are contributing to steelhead mortality. Of the factors, only disease (esp. *Nanopheytus*) had a higher likelihood of contributing to steelhead mortality based on its ability to explain the mortality patterns observed in Puget Sound; therefore, the Workgroup attempted to also list the factors in order of probability.

3.3.1 Disease

Increased pathogen loads may reduce swimming performance or stamina, lead to disorientation, or have other sub-lethal effects that may increase Puget Sound steelheads' susceptibility to predation. Given the amount of time it would take between exposure and effect, it is likely that the pathogen would have to be encountered either in the freshwater or very early in the marine stage. Furthermore, all of the smolts tagged for Hood Canal telemetry studies appeared healthy and in good condition and exhibited no external signs of pathology, and the outmigration rate through Puget Sound (10 day average) is rapid, so any disease would have to impact the smolts within a short period (pers. comm. B. Berejikian 2012).

In July 2013, the Workgroup convened with fish health experts to discuss potential diseases and fish health issues that may be playing a role. The fish health experts' complete report is provided at the end of this document[23], and their findings are summarized below:

Although the characteristics of other pathogens may align with the observed mortality patterns of juvenile steelhead in Puget Sound, based on current knowledge *Nanopheytus salmonica* is the strongest candidate.[24] Steelhead are highly susceptible to *Nanopheytus.* Within the Salish Sea, *Nanophyetus* occurs in high prevalence and intensity among salmonids in the watersheds with the lowest steelhead SARs and highest early marine mortality (south Sound and Hood Canal). High infection intensity among freshwater outmigrants could result in rapid mortality shortly after seawater entry (Jacobson et al. 2008) especially in consideration of decreased swimming performance (Butler and Millemann 1971) accompanied with a predisposition of diseased individuals to predation. Steelhead are most likely exposed to *Nanophyetus* as outmigrating juveniles because the intermediate snail host is critical to establishing piscine infections. The snails reside in riverine and estuarine areas of the watershed, and a parasitic life stage released from the snails is infectious to wild and hatchery steelhead (pers. comm. P. Hershberger 2013).

Other pathogens that are known to be present in Puget Sound and have the potential to cause mortality or lead to sub-lethal effects that result in juvenile steelhead mortality were considered moderate priority for investigation. The moderate priority pathogens include:

- Bacteria: *Listonella (Vibrio) anguillarum, L. ordalli,, Aeromonas salmonicida, Tenacibaculum (Flexibacter) maritimum, Flavobacterium psychrophilum*.
- Harmful Algae Blooms (HABs): *Chaetoceros spp*. (*Heterosigma akashiwo* was not listed by the fish health experts but is also present in Puget Sound and associated with salmon mortality (Rensel 2007)
- Rickettsia: *Piscirickettsia salmonis*.

Finally, those pathogens that have the lowest likelihood of causing direct or sub-lethal effects that result in juvenile steelhead mortality in Puget Sound are:

- Bacteria: *Renibacterium salmoninarum*.
- Viruses: Culturable and emerging viruses.

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[23] Appendix 2: Fish health recommendations to the workgroup

[24] *Nanophyetus salmoincola*, hereafter referred to generically, is a digenean trematode with a complex life cycle involving freshwater / estuarine snails, intermediate fish hosts, and definitive bird / mammalian hosts. The trematode life cycle begins when eggs are expelled into the intestine of fish-eating animals, including raccoons, otters, skunks, coyotes, foxes, herons, merganzers, etc. From the intestines, the eggs pass into the water, where a free-living life stage (miracidium) hatches and penetrates a freshwater or estuarine snail (Oxytrema silicula or Juga silicula). The parasite further develops and multiplies in the snail. Susceptible fish then consume the infected snails; afterwhich a xiphidiocercaria stage penetrates and encysts in the tissues of the fish host. Certain salmonids, including coho salmon, are particularly susceptible to infection. Once in the fish tissues, the xiphidiocercaria transition to a resting stage (metacercaria), the stage most commonly observed in infected fish, especially in the kidney. The trematode becomes sexually mature after infected fish are consumed by a definitive mammalian or avian host, thereby continuing the parasite life cycle (pers. comm. Paul Hershberger, USGS, 2014).

- Parasites/Fungi: sea lice *(Lepeopthirius, Caligus, Argulus spp.), Parvicapsula minibicorbis, Loma salmonae, Nucleospora salmonis, Tetracapsuloides bryosalmonae, Dermocystidium spp., Cryptocaryon spp., Sphaerothecum destruens (Rosette Agent),* and *Ichthyophonus*.

3.3.2 Toxic Contaminants

Contaminant exposure can alter the immune system, either alone (i.e., PBDEs - Arkoosh et al., 2010; Arkoosh et al., 2000; Arkoosh et al., 2001) or in conjunction with other stressors (Jacobson et al., 2003), resulting in an increase in susceptibility to naturally occurring pathogens that cause lethal diseases, potentially leading to population level effects (Arkoosh et al., 1998; Loge et al., 2005; Spromberg and Meador, 2005). Contaminant exposure can also lead to heart defects (i.e., hydrocarbons - Incardona et al. 2006, 2009), disrupt feeding and predator avoidance behaviors (i.e., metals and pesticides - Sandahl et al. 2005, 2007) and have the potential to reduce population productivity (Baldwin et al. 2009). Data on toxic contaminant exposure is lacking for juvenile steelhead originating from Puget Sound; however, juvenile Chinook salmon out-migrating from urban rivers and estuaries of Puget Sound are known to be exposed to PCBs, chlorinated pesticides, PAHs and PBDEs (Stehr et al.2000, Johnson et al. 2007, Olson et al. 2008, Meador et al. 2010, Sloan et al. 2010), often above estimated effects thresholds or at concentrations at which know effects occur. Outmigrating salmon and steelhead may also exposed to less bioaccumulative contaminants including metals such as copper and zinc, typically present in surface runoff from impervious surfaces. Additionally, information is lacking on the extent to which juvenile salmon and steelhead are exposed to newer use pesticides like pyrethroids , and chemical of emerging concerns, including xenoestrogens, pharmaceuticals, personal care products, and. These emerging contaminants have been detected in freshwater streams in Puget Sound and in discharge from waste water treatment plants. It is not yet known the extent to which juvenile salmon and steelhead are exposed to these chemicals, and what effects such exposure might have on long-term survival.

Regional fish toxicologists suspect that marine-derived toxics are likely not a significant contributor to sub-lethal effects resulting in juvenile steelhead mortality. Compared to juvenile Chinook salmon, juvenile steelhead migrate quickly through estuaries and may be less exposed to contaminants present in the urbanized bays of Puget Sound. Furthermore, if marine-derived toxics were contributing to increased susceptibility to disease, it would have to be an incremental effect to describe the slight difference in survival between Puget Sound (toxic) and Hood Canal (not toxic). However, in developed freshwater habitats, steelhead could potentially be exposed to higher levels of contaminants than Chinook salmon. Adult steelhead penetrate further upriver than adult Chinook salmon, often spawning in smaller tributaries, where juvenile steelhead reside for considerably longer periods of time than juvenile Chinook salmon reside in freshwater habitats (Quinn 2005). Small rivers and streams in watersheds with developed landscapes are particularly vulnerable to contaminant input because the volume of contaminated runoff is large compared to the volume of the receiving waters. All salmon species may be exposed in developed fresh water systems; however, steelhead that have a stronger affinity for small streams, may be exposed to higher contaminant levels. However, the Nisqually River is considered a 'reference' (i.e., most pristine stream) for studying the effects of toxics on salmon (pers. comm. N. Scholz, NOAA, 2013) Yet, Nisqually steelhead experience high rates of early marine mortality, even higher than other steelhead populations in Puget Sound.

3.3.3 Genetic basis for reduced predator avoidance

Reduced ability of steelhead smolts to avoid marine predators could have a genetic basis. Potential mechanisms that could lead to a genetic basis for reduced predator avoidance ability include: 1) hatchery-induced selection on locally derived hatchery stocks that influence the natural population, 2) interbreeding between natural populations and out-of-basin segregated hatchery populations, or 3) reduced adaptive genetic variation (caused by hatchery influences or reduced effective population size related [Christie et al. 2012b] or unrelated to hatcheries), or change in the selective regime brought about by a change in the environment (e.g., disease, *sensu* Miller et al., 2011). Consistent with this hypothesis, offspring of naturally spawning hatchery fish have exhibited lower marine (smolt-to-adult) survival than offspring of wild fish in the Kalama River (Leider et al. 1990, see also Araki et al. 2007, 2009 for reduced overall survival of fish with hatchery ancestry), although any number of factors could have contributed to reduced survival of hatchery steelhead offspring. Offspring of resident *O. mykiss* may have lower marine survival that offspring of anadromous *O. mykiss* under certain conditions (Thrower et al. 2009), so increases in the proportion of smolts produced by resident O. *mykiss* could contribute to lower marine survival of natural populations. However, this would not explain the lower marine survival of hatchery populations. Phenotypic traits potentially related to susceptibility to predation may include changes in outmigrant size, outmigration timing, swimming performance or stamina, or susceptibility to disease. Limited evidence suggests that hatchery-induced selection can reduce predator avoidance ability in steelhead during the freshwater phase (Berejikian 1995), and there is a larger body of evidence suggesting that hatchery-induced selection can alter a number of physiological and behavioral traits (reviewed Fraser et el. 2008) that may be related to fitness (Christie et al. 2012a).

The following are some reasons why one may hypothesize a genetic basis for increased predation on steelhead in Puget Sound:

- Hatchery production and outplanting of Chambers Creek steelhead was historically substantial and has occurred throughout Puget Sound since the mid 1950s.
- Wild steelhead population sizes have decreased significantly since the 1980s, which could affect the fitness of subsequent generations.

However, some direct evidence exists that may counter the hypothesis:

- From 1978-1990, Johnson and Cooper (1991) found no evidence of a shift in age or size at outmigration for the Snow Creek wild steelhead population; however, the time period of greater concern is from 1990-present.
- Stocking of Chambers Creek derived steelhead was discontinued in the Nisqually River in 1982.
- Two of the Hood Canal populations monitored for early marine survival (Hamma Hamma and Big Beef Creek) have never received steelhead smolts from Chambers Creek or other segregated broodstocks. Nisqually River and Hood Canal steelhead experience high rates of early marine mortality similar to other populations with greater or more recent hatchery influence (see fig 11).

- There is not substantial variation in early marine mortality among the Puget Sound populations; however, there is substantial variation in population size and the degree of potential hatchery influence among those populations.

3.3.4 Foraging/Starvation

A decrease in prey abundance or quality or the altered distribution of prey could lead to changes in foraging behavior (e.g., different habitat use, greater searching, and vertical distribution). An increase in foraging behavior could make steelhead more susceptible to predation. Starvation could also make steelhead more lethargic or compromise the immune system increasing their susceptibility to disease and predation. However, the rapid rate of migration through Puget Sound and absence of lateral movements suggest steelhead are not focused on foraging (based upon the telemetry data). Food supply may play a role in community structure and affect steelhead to the extent that it affects predator or 'buffer prey' abundance.

3.3.5 Outmigration timing

Telemetry data have not indicated strong timing effects on survival in Hood Canal (pers. comm. B. Berejikian, NOAA, 2013). Furthermore, there is no correlation between outmigration timing and survival of Snow Creek smolts from 1978-1990 (Johnson and Cooper 1991). However, this is prior to the period of substantial decline in Puget Sound steelhead SARs, and Snow Creek enters the Strait of Juan de Fuca and is not representative of inner Puget Sound and Hood Canal populations, where the decline in SARs has been most apparent. Outmigrant timing among populations appears to primarily be related to winter stream temperature (Figure 22).

3.3.6 Outmigrant size and early marine growth

There may be signs of size-selective mortality (bigger is better) occurring while steelhead from Puget Sound are in the marine environment as a whole (Ward 2000 and Thompson et al. unpublished 2013. However, there is no evidence of size-selective mortality regulating survival while steelhead are in Puget Sound as juveniles. Survival models that include data from 9 (acoustic tagged) populations encompassing Puget Sound and Hood Canal show a weak negative correlation between body size and estimated survival. The absence of a positive relationship is supported by greater tag burden for smaller fish (Moore et al. 2010 and unpublished data). These studies include tagged migrant steelhead over a body mass range of 30 to 90 grams, which excludes only the bottom 10% of the total wild smolt size-frequency distribution. Even very high growth rates of 1% body mass gain per day would equate to an average size smolt of 45 g, increasing to a body mass of 52 g during the two week residence in Puget Sound. Furthermore, there was no evidence of a shift in age or size at outmigration of Snow Creek steelhead from 1978-1990 (Johnson and Cooper 1991).

No size-selective mortality was found in a similar acoustic telemetry study performed in the Strait of Georgia (Melnychuck 2007). Furthermore, another study performed in the Strait of Georgia by Friedland et. al. (2013) found that, between 1977 and 2005, smolt size at ocean entry (length) was a good predictor of smolt-to-adult survival rates at the beginning of the time series (through the late 1980s). However, as smolt-to-adult survival rates declined (1991-2005), it became apparent that size at ocean entry was no longer capturing the variability associated with the marine environment.

3.3.7 Physiological issues during smoltification

The telemetry data are not consistent with physiological issues such as stunting and parr reversion. If that were the case, one would expect the steelhead to delay entry into the marine environment or return to the freshwater. One also may expect to see size-selective mortality occurring, with smaller, less developed fish performing more poorly (section pers. comm. P. Swanson, NOAA, 2013).

3.3.8 Habitat modification/simplification

Based upon the telemetry data, steelhead smolts show a tendency to migrate in open water, away from nearshore areas. Their brief residency in Puget Sound suggests a minimal role of nearshore structural habitat (eelgrass, kelp forests), but these habitats may be important for community structure and affect steelhead to the extent that it affects predator or 'buffer prey' abundance, or foraging behavior and associated predation risk.

3.4 What's next?

The Workgroup concluded that the best approach to reducing uncertainty regarding the potential role of fish condition and/or altered behavior is to begin by performing broad, coarse studies and to use the result of those studies to determine whether and in what direction next steps are warranted. These include retrospective studies correlating existing data on environmental and steelhead individual and population characteristics with SARs and telemetry results, a genome-wide association study (GWAS) to discover genomic differences between outmigrating steelhead smolts that survived to open ocean entry (exiting Strait of Juan de Fuca) versus those smolts that died somewhere within the Salish Sea (using DNA collected before releasing acoustic tagged smolts in 2006-2009), and a reciprocal transplant study that will help determine whether early marine mortality is associated with environmental or genetic effects in the freshwater vs. environmental conditions in the Puget Sound marine environment. A fish health assessment will also be performed, focusing on evaluating the prevalence and intensity of *Nanopheytus* throughout Puget Sound and determining whether contaminant exposure could be exacerbating fish health issues in certain regions of Puget Sound. Gut contents and tissues will also be preserved for evaluating diet composition and the presence of other pathogens, respectively, if it is deemed valuable in the future.

As it is currently considered a moderate priority, the fish health assessment will not include a direct investigation for disease effects associated with HABs (based upon the fish health experts' assessment and because one may expect more inter-annual variation in survival and population x year interactions if HABS play an important role given the variability in bloom timing). However, the Workgroup will look for existing documentation regarding the presence, intensity and timing of HABs (*Chaetoceros spp.* and *Heterosigma a.*) that could be reviewed to determine whether there are any correlations with steelhead SARs and early marine mortality.

Overview of the Research Components

The following set of research components are intended to improve our answers to the three questions that constitute the framework of this work plan (see page 5). The focus continues to be on better isolating where, when and at what rate mortality is occurring, determining who the potential predators are, and determining whether other mechanisms beyond increases in potential predator abundance are fundamentally affecting steelhead survival in Puget Sound. Existing data will be evaluated to better support or refute factors that have a moderate probability of contributing to mortality (or where high uncertainty exists). Field research will be conducted to better identify the locations, rate and timing of mortality; determine whether the causes of mortality are freshwater - or marine-derived; and better understand the factors that current evidence suggests are likely contributors to juvenile steelhead mortality. The work will build upon the evidence described in the previous section toward ultimately determining the primary causes of steelhead mortality in Puget Sound. See Appendix B. for a complete description of each study.

The study results will be comprehensively evaluated by the Workgroup as a whole, and will be presented to outside experts in aggregate for review and discussion. This is discussed in the Project Management section, below.

Study 1: Complete the Puget Sound-wide analysis of acoustic telemetry data

The Puget Sound-wide analysis of acoustic telemetry data will be completed, describing juvenile survival, migration rates and patterns, mortality by distance traveled and by time in the saltwater, hatchery/wild survival, and potential mortality hot spots (Moore et al. NOAA).

Study 2: Assess existing SAR data for trends

The assessment of declining trends in SARs, which primarily reflect marine survival, will be completed for both hatchery and wild summer- and winter-run steelhead in Puget Sound. The differences and similarities in trends in SARs will be compared with other neighboring regions. The variability of trends among Puget Sound populations will also be evaluated. This population-specific assessment is vital for determining the spatial and temporal scales of the problem and will provide the foundation for correlative analyses with factors potentially affecting survival.

Study 3: Evaluate smolt characteristics and population life-history data vs. SARs

Existing data on wild and hatchery steelhead individual and population characteristics will be analyzed with reference to SAR trends over time. Ideally, this effort would also include developing new data collection stations for additional Puget Sound and Salish Sea populations into the future. The goal of this work will be to evaluate whether a loss of population life-history diversity is associated with declining SAR rates and examine hypotheses concerning spatial variation in mortality, size-selective mortality, match-mismatch, and life history variation. This analysis will also look at correlations between changes in hatchery and wild smolt outmigrant abundance and SARs.

Study 4: Compare SAR and telemetry data on marine mortality with environmental and buffer prey data

Puget Sound steelhead SAR trends and mortality data obtained from telemetry studies will be compared to existing and to-be-collected environmental and potential buffer prey data to determine whether correlations exist. Environmental data would include Puget Sound temperature, turbidity, pathogens, contaminants, and harmful algal blooms (HABs). Buffer prey data will include abundance, size and age composition data for herring (and other prey alternatives if data are available).

Study 5: Identify juvenile steelhead predators in the marine environment

The literature review to identify the most likely predators on steelhead smolts will be completed. For all piscivorous birds and mammals in the region that are reasonably abundant during steelhead outmigration, we will determine: 1) Does the predator eat juvenile salmon?, 2) Does the predator eat juvenile steelhead?, 3) Does the predator feed at the depth steelhead typically outmigrate?, 4) Is there an overlap in size distribution between predator diet and that of juvenile steelhead (especially in mortality hotspots)? 5) Is the predator reasonably abundant in Puget Sound and Strait of Georgia (especially in steelhead mortality hotspots) in May and June when steelhead typically outmigrate?, and 6) Is the predator population increasing and/or has there been a change in distribution during the time of steelhead decline? A cursory review of piscine predators will also be integrated into this assessment, using the same evaluation format. The piscine predator data is considered more cursory due to the more limited information on diets and population trends.

Study 6: Perform a GWAS study of acoustic tagged steelhead smolts

An investigation will be performed to determine whether there is an association between genomic signatures and survival in outmigrating steelhead smolts in the Salish Sea. A genome-wide association study (GWAS) will be performed to discover genomic differences between outmigrating steelhead smolts that survived to open ocean entry (exiting Strait of Juan de Fuca) versus those smolts that died somewhere within the Salish Sea (using DNA collected before releasing acoustic tagged smolts in 2006-2009). GWAS use genome scans (e.g., a panel of 100s to 1000s of single nucleotide polymorphism, SNPs) to document a relationship (i.e., association) between a phenotype (e.g., morphological feature, behavior, illness, measure of fitness or survival) and a genotype (e.g., blocks of linked SNPs), based on population samples.

Study 7: Conduct a Puget Sound-wide fish health assessment of outmigrating juvenile steelhead

A field assessment of juvenile steelhead health as they migrate from fresh water habitats through the marine systems of Puget Sound will be conducted to detect signs or symptons of disease that may impair their survival in Puget Sound. The assessment will target up to five watersheds—Nisqually, Green/Duamish, Snohomish, Skagit and Tahuya—and major marine basins in Puget

Sound. Steelhead will be collected and compared in three general locations: 1) the freshwater environment (hatchery release and in smolt traps), 2) the estuary (using fyke net locations, beach seines, and 3) the marine environment (purse seines).

This assessment will focus on the infection prevalence and intensity of *Nanophyetus* in cohorts of outmigrating steelhead. Assuming enough juvenile steelhead can be captured in the marine environment, some indication of whether the disease leads to mortality in infected outmigrants will be provided by examining the progression of infection prevalence and intensity (by histology) as the smolts out-migrate.[25] If the results support the hypothesis that infected individuals experience elevated mortality, then additional controlled studies (e.g., live box challenges, laboratory disease challenge) will be recommended to address cause-and-effect. Contaminant exposure will also be assessed to determine whether contaminants are exacerbating pathogen prevalence and intensity. Tissue residues will also be compared with published effects thresholds to evaluate the potential health effects on juvenile steelhead from exposure to contaminants. Finally, gut contents and tissues will be preserved for evaluating diet composition and the presence of other pathogens, respectively, if it is deemed valuable in the future.

We will collaborate with other fish collection efforts to reduce costs and increase coverage, and with the proposed *Nanopheytus* study of coho (part of the Salish Sea Marine Survival Project).

Study 8: Perform a reciprocal transplant study to partition population or river of release effects from Puget Sound location effects

The Puyallup, Green and Nisqually populations all show steeper declines in survival in Central Puget Sound than in other segments of their migrations. In particular, the Nisqually smolts experienced higher survival from the estuary to the Tacoma Narrows Bridge than Green River smolts from the Duwamish estuary to the next telemetry line in Central Puget Sound. Nisqually smolts experienced lower survival from Tacoma Narrows to Central Puget Sound than in any other segment earlier or later in their migration. This suggests a mortality hotspot in Central Puget Sound. The higher initial marine mortality of Green River steelhead than Nisqually steelhead may be caused by: 1) rearing environment effects or genetic effects on individual fish condition (freshwater derived), or 2) differences in the initial environmental conditions experienced by smolts migrating through south vs central Puget Sound (marine derived).

A reciprocal transplant study will be performed to evaluate this, where fish from both the Green and Nisqually rivers are acoustically tagged and released from sites in both the lower Green and Nisqually rivers. We will install acoustic receiver lines at each river mouth, 25 km North of each river in South and Central Puget Sound, and the existing Admiralty Inlet line will have new batteries installed. The fish health assessment described above will be coordinated with this effort to determine whether mortality correlates with fish health. The translocation effect (translocated or

[25] *After the draft work plan was completed, the Steelhead Workgroup decided to add a laboratory as a backup measure for evaluating the potential effects of nanopheytus as steelhead travel into the marine environment. The backup measure was added due to concerns about the number of steelhead that will feasibly be captured in the estuary and marine environments. See the complete write-up in Appendix 1 for more information.*

not) while not necessarily of interest, can be accounted for in this study. This study will also improve hotspot identification.

Study 9: Evaluate interactions between harbor seals and steelhead smolts in Puget Sound

This exploratory study intends to quantify spatial and temporal overlap of harbor seals and steelhead smolts in Puget Sound and potentially document predation through the use of acoustic telemetry and other tagging methods. Direct observation of predation on salmon or steelhead smolts are rare, and would be impractical, difficult, and costly to conduct. Harbor seals and harbor porpoise are known to eat steelhead smolts (or fish of the same size) and have exhibited abundance increases over the period of steelhead abundance declines (Jeffries et al. 2003, B. Hanson pers. comm. 2012). Given the population sizes of the marine mammals compared to the relative total abundance of steelhead smolts, even if steelhead constitute a small percentage of the harbor seal and harbor porpoise diet, their predation may still represent a significant source of steelhead mortality. Because of this, diet composition studies (e.g., scat analyses) may not be fruitful. Concurrent telemetry tagging of steelhead and potential predators provides an opportunity to quantify spatial and temporal overlap and estimate encounter rates. Of the two predators, it is more feasible to evaluate harbor seals because harbor porpoises are difficult to capture and tag.

Harbor seals will be fitted with transceivers to identify their locations before during and after the steelhead outmigration period, foraging times and durations, foraging range from haul outs, and encounter rates (spatial and temporal overlap and direct predation) with steelhead. Encounter rates will be determined by evaluating the proximity between the harbor seals and outmigrating steelhead smolts affixed with acoustic tags. As a secondary benefit to this study, introducing more acoustic telemetry receivers into Puget Sound will also bolster acoustic receiver line efficiency estimates, improve hotspot identification, and provide more information about steelhead migration behavior.

Depending on results from the first year of concurrent tagging of steelhead and seals, other predators (e.g., harbor porpoise) may be investigated or additional year(s) of harbor seal tagging may be required. In conjunction with this research, the Workgroup will also investigate the potential use of retrievable critter cams mounted on harbor seals or harbor porpoises to detect what they are eating. The Workgroup is also following an investigation of new PIT tag technology in Canada that may increase the power of this steelhead encounter evaluation.

Study 10: Test the dinner bell hypothesis

Acoustic pings from telemetry transmitters are within the auditory range of marine mammals. A previous study indicated that acoustic-tagged adult salmon migrating in the Columbia River may have suffered greater mortality than dummy-tagged salmon (pers. comm. M. Rub, NOAA, 2012). To determine whether pinging telemetry tags make steelhead smolts more vulnerable to marine predators, a portion of the transmitters used in the reciprocal transplant experiment will be programmed to be silent during the first week of seawater migration and subsequently activate. The study will compare the detection rate at the Strait of Juan de Fuca receiver array of tags that will ping continuously from the time fish are released to the detection rate of tags that will be silent during the first week of marine migration.

Study 11: Develop ecosystem and bioenergetics models (affiliated work via the Salish Sea Marine Survival Project)

Ecosystem and predator-specific bioenergetics models will be considered for evaluating the effects of various factors on a Puget Sound-wide scale and for evaluating cumulative effects of multiple factors. This activity will be performed in conjunction with model development for evaluating other species via the Salish Sea Marine Survival Project. Ecosystem models that have been discussed include Ecopath with Ecosim and Atlantis. . Additionally, population dynamics demographic models, such as Integral Projection Models (Coulson 2012), will be considered. Also, USGS has proposed to develop a predator-specific bioenergetics model for herring which may be transferrable to steelhead (USGS 2012).

Other studies considered

Evaluate marine mammal and bird predator abundance

A targeted evaluation of marine mammal and bird predator abundance within the steelhead outmigration time period was discussed. However, the group considered it too cost prohibitive to implement relative to the value (very difficult to relate predator abundance to steelhead mortality without additional information about the predator-prey relationship since steelhead abundance is low relative to other prey available) and concluded that related information, while not targeted on the steelhead outimigration window, could be gathered from proposed or ongoing studies. For example, WDFW completed harbor seals surveys in late July and early August of 2013 (pers. comm. S. Jeffries, WDFW, 2013). This data will be used to update the harbor seal abundance estimate for Puget Sound. The results of this type of work contribute to correlative analyses and bioenergetics and ecosystem modeling.

Controlled experiments

Captive, controlled experiments were discussed for evaluating environmental factors that are more difficult to assess in a natural setting. For any controlled experiment, sub-lethal effects could be evaluated by tracking the survival of tagged fish after exposure. The following were discussed:

- Disease challenges. In consultation with fish health experts, the Workgroup concluded that disease challenges may be useful to have a better understanding of the impact of certain pathogens or parasites once the fish health assessment is complete.
- Evaluating the effect of filtered vs. unfiltered seawater on steelhead survival. The Workgroup concluded that a general marine quality assessment was not valuable after discussing the approach with experts.
- Evaluating the effect of time in the Puget Sound marine environment and the general physiological response to seawater by holding tagged fish in seawater for a period of time and then tracking their survival after release. Supporting experts concluded the telemetry data are not consistent with physiological issues such as stunting and parr reversion; therefore, this is not warranted (pers. comm. P. Swanson, NOAA, 2013).

Evaluating predation via predator diet composition

Predator scat or gut content analyses(for presence/absence and diet composition) were discussed but considered of low value since it would be difficult to gauge predator impact in this manner. Juvenile steelhead abundance is low relative to other prey available. However, members of the Workgroup are in the process of determining whether we can collaborate with a broader study proposed for the entire Salish Sea to evaluate the impact of harbor seals on juvenile and adult salmon using DNA quantification of prey in scat.[26]

Barging (or trucking) studies

Several study concepts utilizing barges were discussed. They included:

1. Barging acoustic tagged steelhead past mortality hotspots, releasing them, and comparing the survival of these versus steelhead tagged and released in the lower river.
2. Barging an entire hatchery smolt release group (or a coded-wire tagged group) and evaluating SARs relative to an unbarged/river release group.
3. Barging steelhead through their natural migration route, comparing the effects of filtered vs. unfiltered water to determine whether water quality (disease, toxics, temperature, etc) affects the health of outmigrating smolts. Sub-lethal effects would be evaluated by releasing portions of acoustic tagged fish at critical points along the migration route. A river release group of tagged steelhead would be included and used to serve as a baseline for the barge group and compare hatchery and wild to estimate the relative performance of the hatchery fish as surrogates for wild.

Barging effects were a significant concern of the Workgroup. For example, barged fish could be disoriented upon release, leading to increased mortality, and there is a significant concern about straying if a large number of steelhead are barged and released as is proposed in concept 2 (Keefer and Caudill 2012, Keefer et al. 2008, and Solazzi et al. 1991). Any barging study would need to result in increased survival and, even then, it would be impossible to determine whether barging effects still occurred and survival should be higher. However, a barging study similar to concept 1 was performed in the Seymour River of the Strait of Georgia that resulted in increased survival of steelhead barged through (to bypass) an estuary prior to release (Balfry et al. 2011). Potential barging effects aside, barging study concepts 1 and 2 do not help isolate the causes of mortality and would be more relevant as an alternative to identifying mortality hotspots. However, hotspots have been identified and will be refined (via the reciprocal transplant study) without the use of barging.

Evaluating growth after seawater entry

The evaluation of growth differences after seawater entry (using otoliths or scales) at critical life stages was also discussed. Puget Sound could be compared with coastal/lower Columbia River populations and shifts in growth within populations over time could be evaluated. This information

[26] This research is being proposed by Austen Thomas (University of British Columbia) and Alejandro Acevedo-Gutierrez (Western Washington University).

could be correlated with SARs to determine if differential growth is leading to differential survival. While this appears to be cost effective and compelling research, there is no evidence that growth during the limited time steelhead are in the Puget Sound marine environment is critical. Furthermore, it may be difficult to evaluate growth during the short time period steelhead are in Puget Sound. Finally, the amount of otoliths available may be limited. However, this study was recommended in the Salish Sea Marine Survival Project, Puget Sound Research Proposal (incomplete, not released) for multiple species, and steelhead could be included.

Evaluating the portfolio effect using ecological genomics

The portfolio effect could be evaluated to determine whether a loss of population and life-history diversity is negatively affecting steelhead survival. This effort would use ecological genomics to compare genomic effects of Puget Sound vs. coastal populations, and determine whether the effects are associated with run-timing and/or hatchery introgression. This effort was initially proposed to Washington Sea Grant in 2013 by Seeb (UW) and Warheit (WDFW); however, it was declined. The Workgroup concluded that the higher level of funding required to perform this type of effort would be better spent evaluating other factors.

Evaluate predation rates with PIT tags

PIT tagging/retrieval studies were also discussed to evaluate predation by birds. However, the preliminary results indicate that marine mammals are likely the primary predators. For marine mammals (e.g., harbor seals), a large number of scats would need to be sampled during the short steelhead outmigration window to detect PIT tags, and there are only a few places in Puget Sound (e.g., Protection Island, Dosewallips River estuary) where this may be feasible (pers. comm. S. Jeffries, WDFW, 2013). The Workgroup will continue to investigate prototype PIT tag detection technologies that may be useful for evaluating mammal encounter rates.

Long-term needs, broader than scope of work

The Workgroup identified other information needs outside the scope of this effort but relevant to steelhead recovery. They include:

- Increase the collection of wild steelhead fish-in, fish-out[27] data spatially and temporally throughout the Puget Sound (and the rest of the Salish Sea) to improve our understanding of trends and variation in marine and freshwater survival relative to individual and population characteristics.
- Perform work to improve our understanding of the anadromous/resident relationship.

[27] Fish-in, fish-out = spawner abundance and juvenile/smolt outmigrant abundance, typically performed via redd surveys and smolt outmigrant trapping.

- Assess hatchery introgression issues and how this has affected age structure, outmigration, spatial structure, etc. across Puget Sound populations.
- Learn more about the high seas distribution of Puget Sound (and the greater Salish Sea) steelhead versus other regions. Consider study approaches such as analyzing carbon isotopes in adult returns relative to sea surface temperatures to develop a migration pattern, as described in MacKenzie et al. 2011. Also assess Puget Sound steelhead entry timing into the Pacific Ocean compared to other regions. And, compare isotopic differences between healthy and weak stocks to test if they use different oceanic regions.
- Assess predation of adult steelhead in Puget Sound (and throughout the Salish Sea).

Project Management, Coordination, Outreach, and Communications

The Workgroup will continue to utilize the project management, coordination, outreach and communications infrastructure of the overarching Salish Sea Marine Survival Project. This will be complemented by WDFW's own outreach and communications capacity.

Project management, coordination

WDFW will lead the implementation of the work plan through an interagency agreement with the Puget Sound Partnership, and the effort is coordinated by LLTK. As a collaborative effort directly involving NOAA Fisheries, the Nisqually Tribe, the Tulalip Tribes, the Northwest Indian Fisheries Commission, US Geological Survey, Seattle City Light, and others, the Workgroup will continue to convene over the course of the study period to plan and implement the research, discuss its outcomes, and determine on what path to continue. Meetings will occur bi-monthly, or more as needed. A project management web site will be established to maintain the research work schedule, communicate regarding activities, store and manage data, etc.

The Workgroup will coordinate with the Salish Sea Technical Team on overlapping research, research outcomes and next steps. The Workgroup will also periodically report to the Salish Sea Coordinating Committee on progress and will discuss with the Coordinating Committee potential management strategies for addressing various factors should the research indicate that they are likely responsible for the high early marine mortality rates in Puget Sound.

LLTK and the Workgroup members will work with NOAA Fisheries and the Puget Sound Partnership to ensure that the current perspectives discussed in this document and the subsequent research outcomes inform the ongoing Puget Sound steelhead recovery planning process. Under the Salish Sea Marine Survival Project, LLTK will also continue to coordinate this research with the efforts of the Puget Sound Partnership's Puget Sound Ecosystem Monitoring Program. Finally, periodic reports will be provided to the Puget Sound Recovery Implementation Technical Team and the Puget Sound Science Panel who have identified this work as a priority in their 2011-2013 Science Plan.

The results of all the studies in the work plan will be comprehensively evaluated by the Workgroup as a whole and will be presented to outside experts in aggregate for review and discussion. This will be led by the Washington Department of Fish and Wildlife project manager, Neala Kendall, and project coordinator, Michael Schmidt of Long Live the Kings. A series of workshops will be held to disseminate the results and discuss them in aggregate under the umbrella effort, the Salish Sea Marine Project. Also , sessions summarizing the research results will be hosted at conferences or workshops such as American Fisheries Society Conferences, the Salmon Ocean Ecology Workshop, and the biennial Pacific steelhead management meeting hosted by the Pacific States Marine Fisheries Commission.

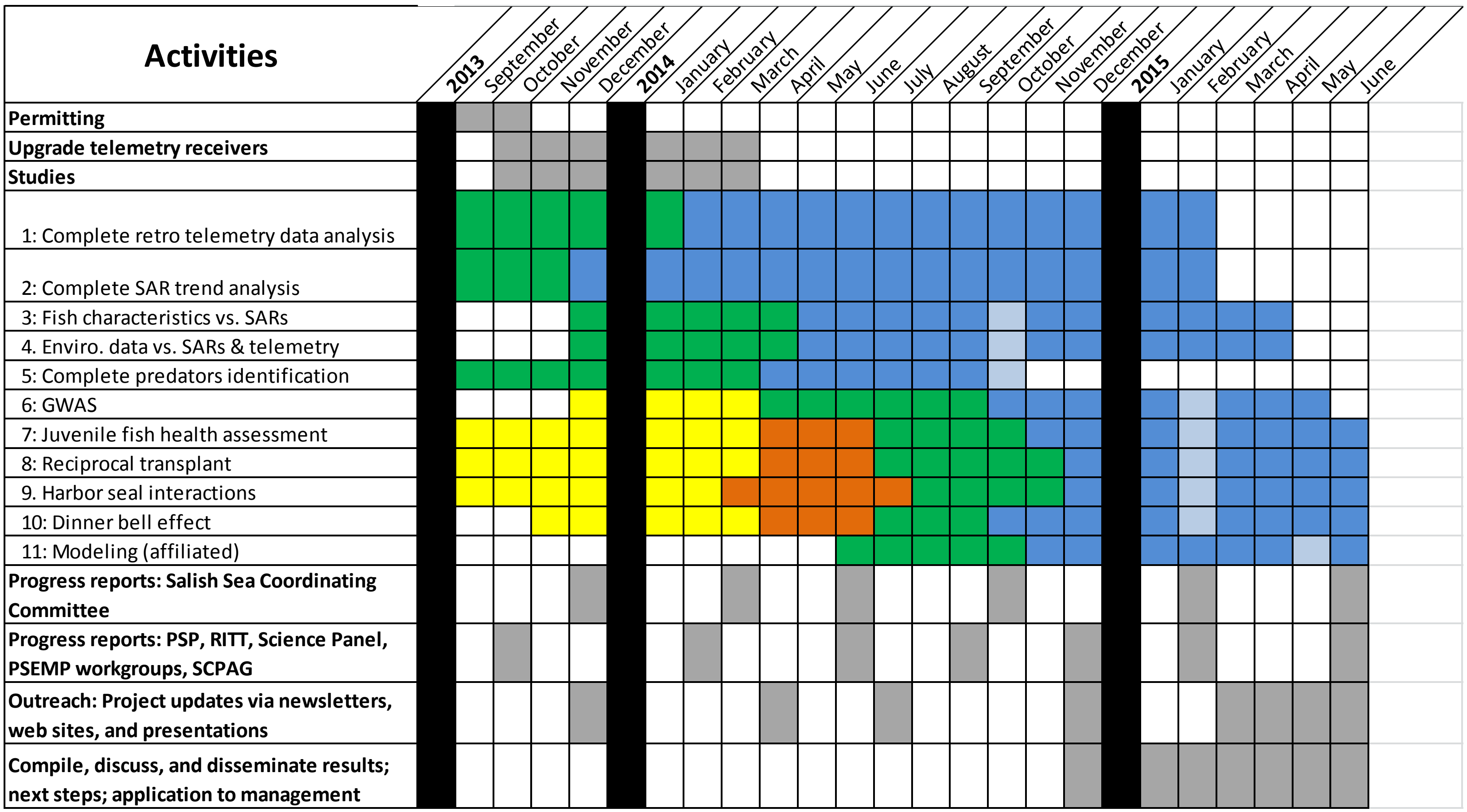
See the Work Schedule section for more information.

Outreach and communications

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

Work Schedule

The following diagram describes the workflow. Coordination and outreach activities are included to describe how progress and results will be communicated. Study timeframes include: preparation, field work, analysis, reporting, technical memo due. Progress reports for all studies will be due in June 2014 and December 2014. All work must be completed by June 30th, 2015.



Budget

The following is a general budget. The total cost of the effort is $1.65 million dollars. Approximately half of that is provided as match (wages and equipment) by the participating entities.

Costs		Studies
Analyze existing data for trends and correlations with a range of potential contributing factors	$50,000	1: Telemetry data analysis, 2: SAR trend analysis, 3: Fish characteristics vs. SARs, 4. Environmental data vs. SARs and telemetry, 5: Predators identification,
Perform laboratory and field studies to narrow the field of contributing factors	$830,000	6: GWAS, 8: Reciprocal transplant
Perform targeted field studies of contributing factors evidence currently point to	$700,000	7: Juvenile fish health assessment, 9. Harbor seal interactions
Project Coordination, Outreach, Communications	$70,000	Project coordination, communications, permitting, meeting facilitation, etc.
Total	**$1.65 million**	
Revenues		
State Appropriation	$788,000	
Match	$832,000	NOAA, WDFW, Tulalip Tribes, Nisqually Tribe
Other (pending)	$30,000	
Total	**$1.65 million**	

*Costs associated with testing the dinner bell hypothesis (study 10) are incorporated in the reciprocal transplant study (8). Costs associated with modeling (study 11) are not included as they are currently not funded and affiliated with other efforts, but a rough estimate for the ecosystem and bioenergetics modeling is $350,000 for salmon and steelhead.

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Figures

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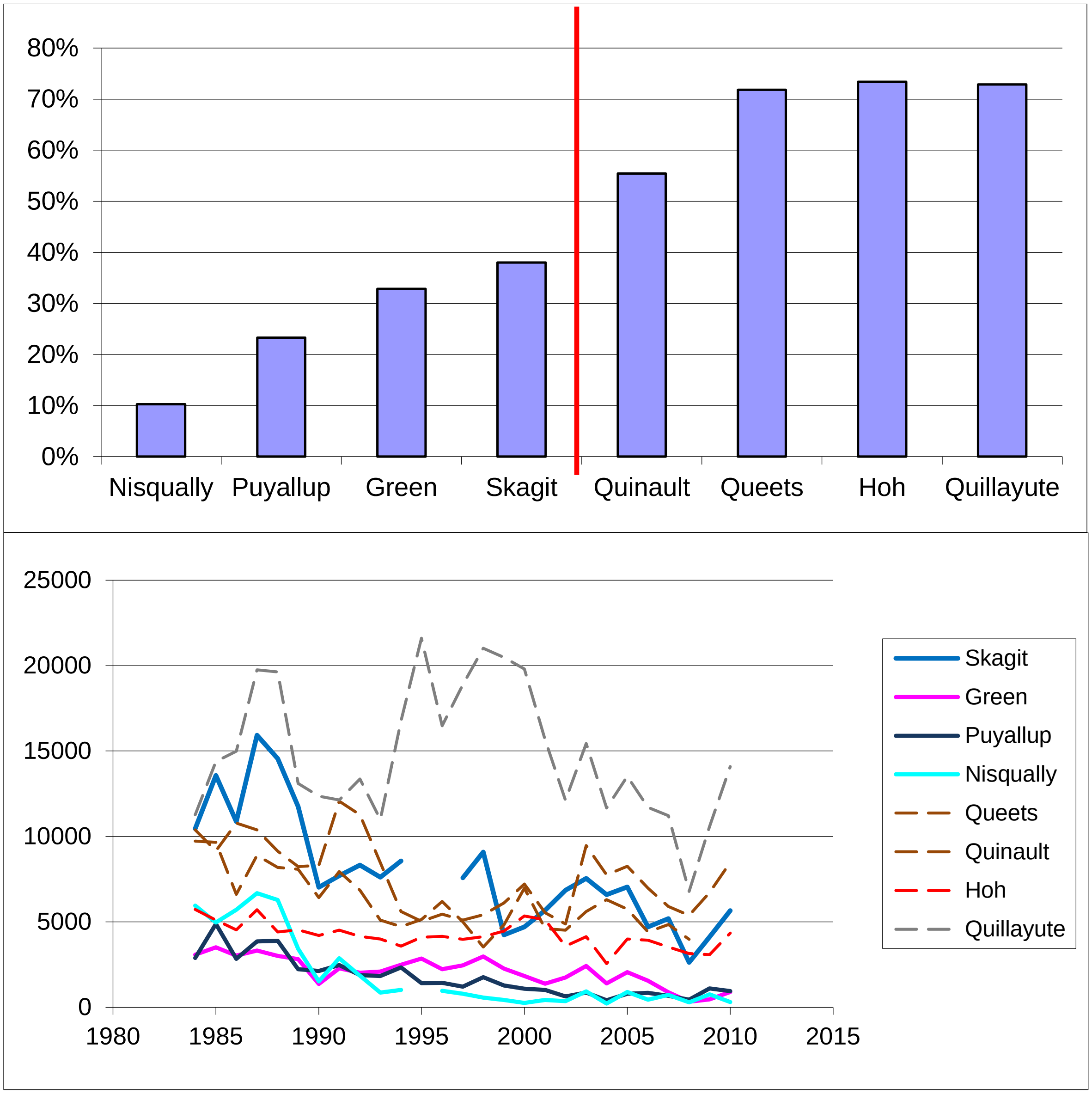


Figure 2. Top panel is the average adult wild steelhead abundance for the most recent six run years (2005-2010) divided by the average adult abundance for run years 1984-1989. Populations are listed from south to north within Puget Sound and the Washington Coast. Bottom panel shows individual population trends by year and are generated from the same data. Dashed lines represent the Washington coastal populations and solid lines represent Puget Sound populations. (unpublished data provided by Bob Leland (WDFW) via Ken Warheit (WDFW) 2013)

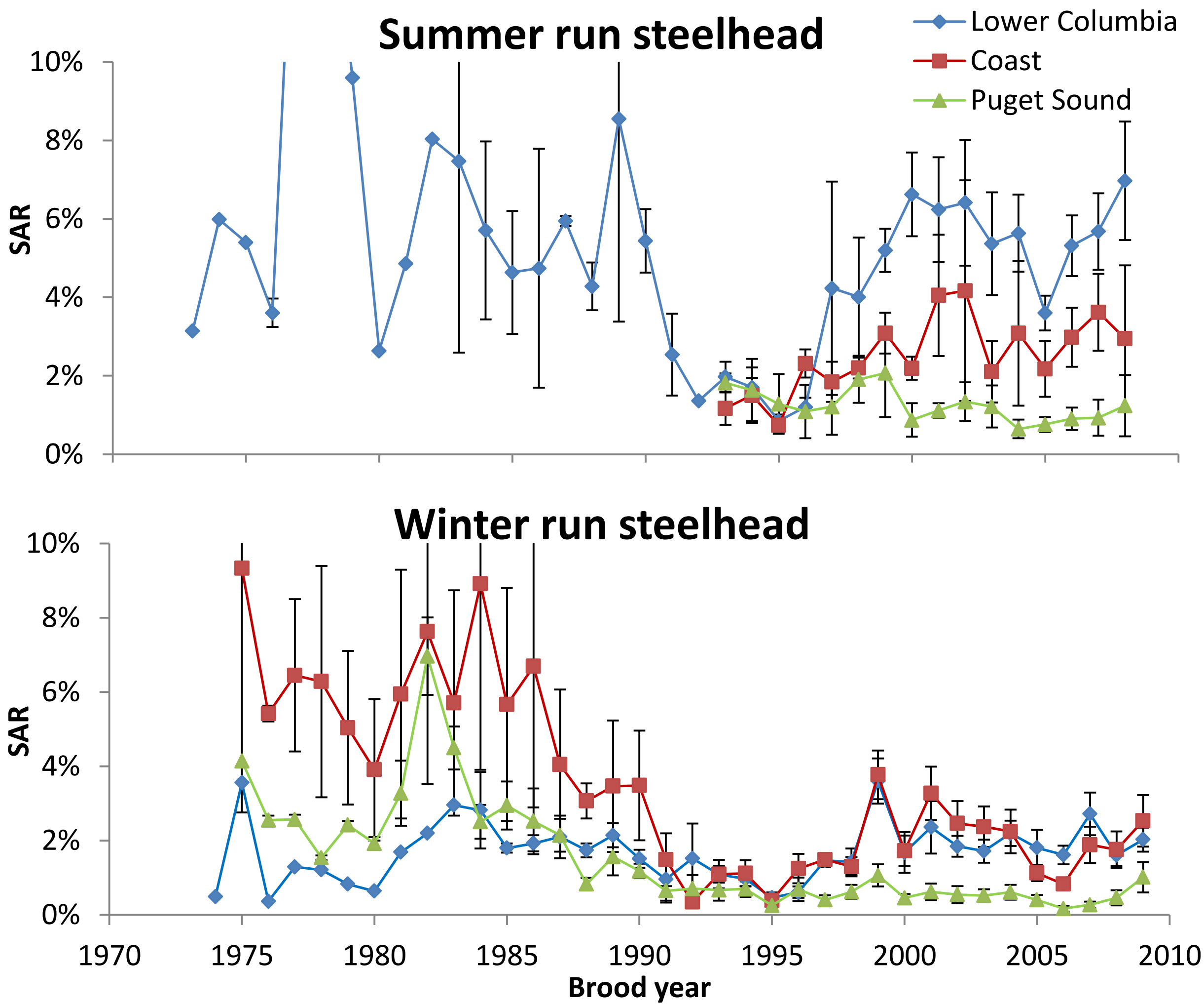


Figure 3. Average marine (smolt-to-adult) survival rates for hatchery summer and winter run steelhead, comparing Puget Sound to the Lower Columbia River and Washington Coast. Averages do not include all hatchery stocks within a region because of the lack of data (produced by Kendall et al., WDFW 2013 unpublished data. See Appendix A for the list of stocks used to create these Figures).

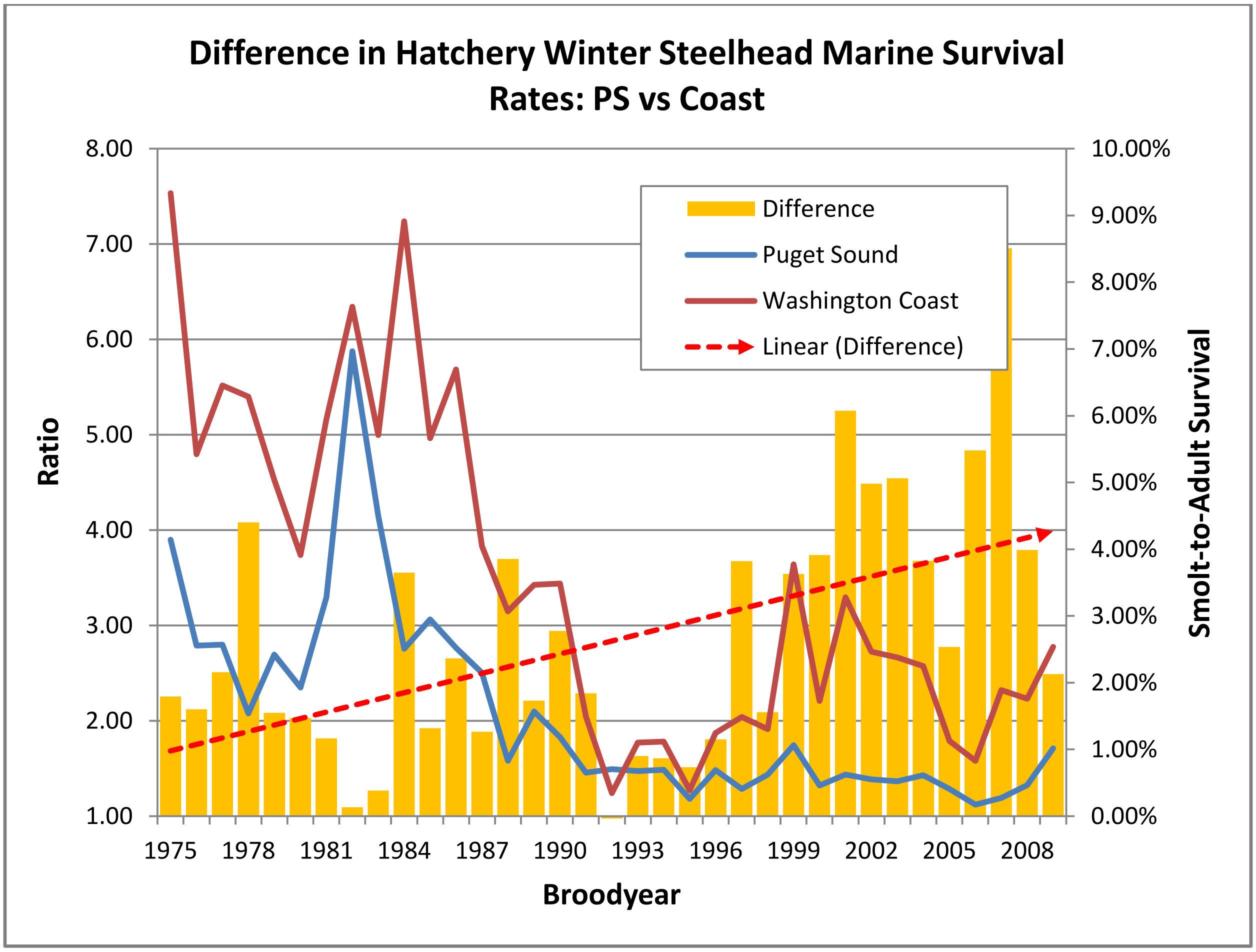
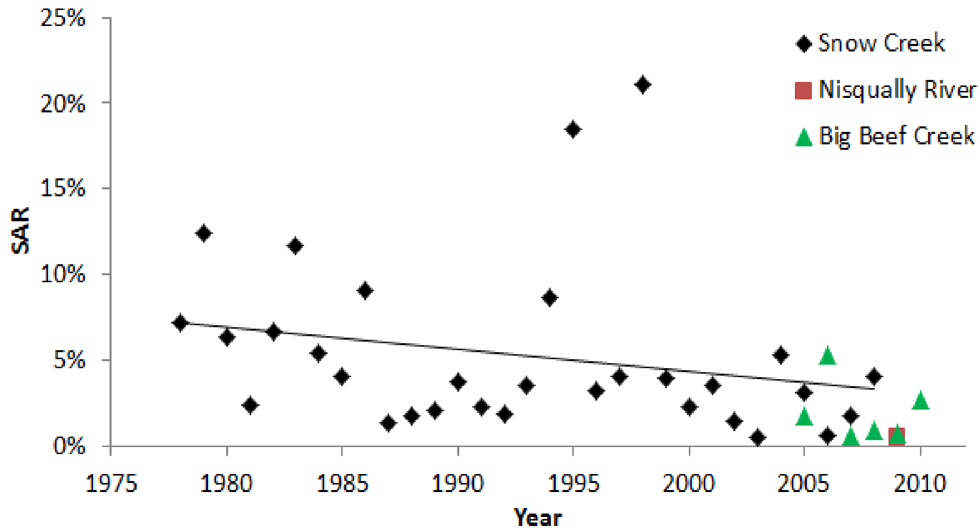


Figure 4. The difference between Puget Sound and Washington Coast marine (smolt-to-adult) survival has increased, one potential indication of increased mortality in Puget Sound (produced by Schmidt, LLTK, using the Kendall et al., WFDW, 2013 unpublished data. See Appendix A for the list of stocks used to create these Figures).

Wild steelhead trends in Puget Sound



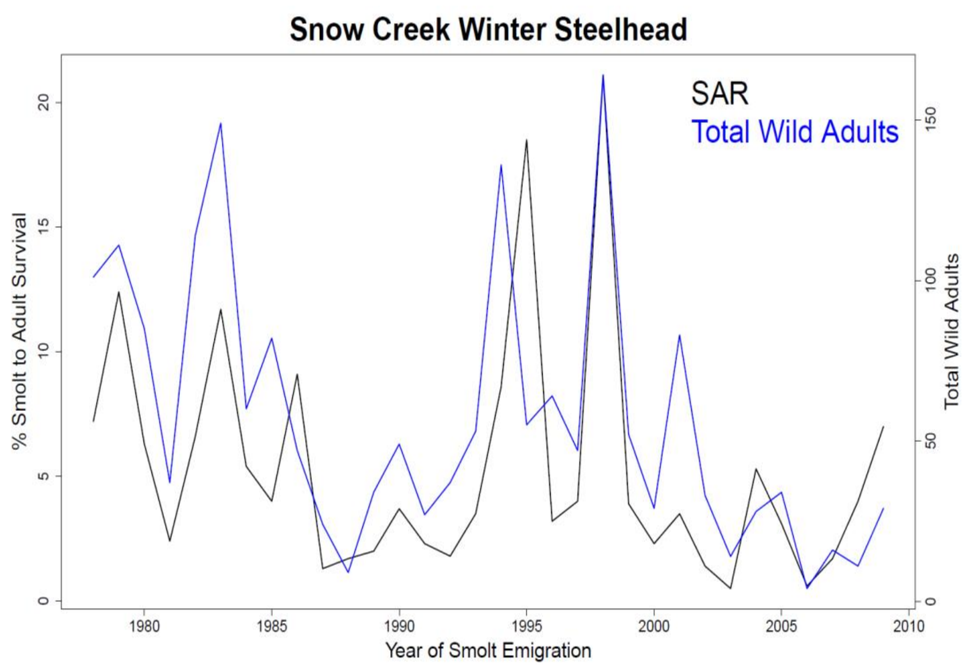


Figure 5. Top panel**,** SARs for Snow Creek, Big Beef Creek, and the Nisqually River, combined (produced by Kendall et al., WDFW 2013 unpublished data). Bottom panel, SARs and adult return abundance trends for Snow Creek (produced by Kemp, LLTK, using WDFW 2012 unpublished data).

Hatchery trends within Puget Sound

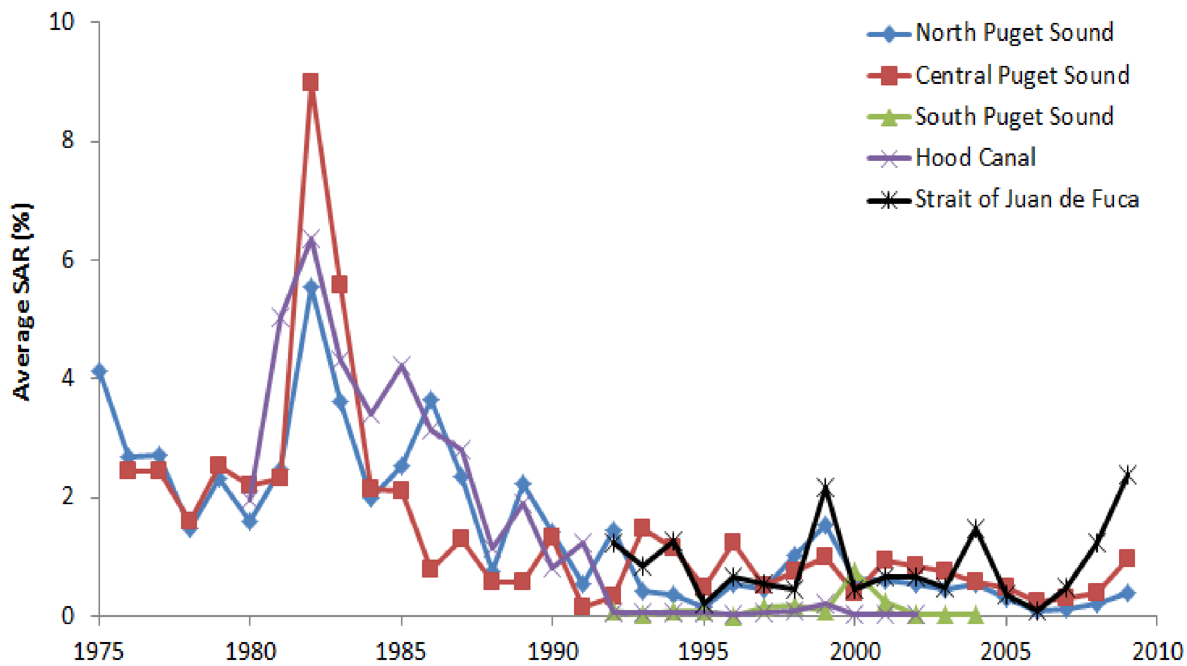


Figure 6. Hatchery winter steelhead smolt-to-adult survival trends in Puget Sound (produced by Kendall et al., WDFW 2013 unpublished data. See Appendix A for the list of stocks used to create these Figures).

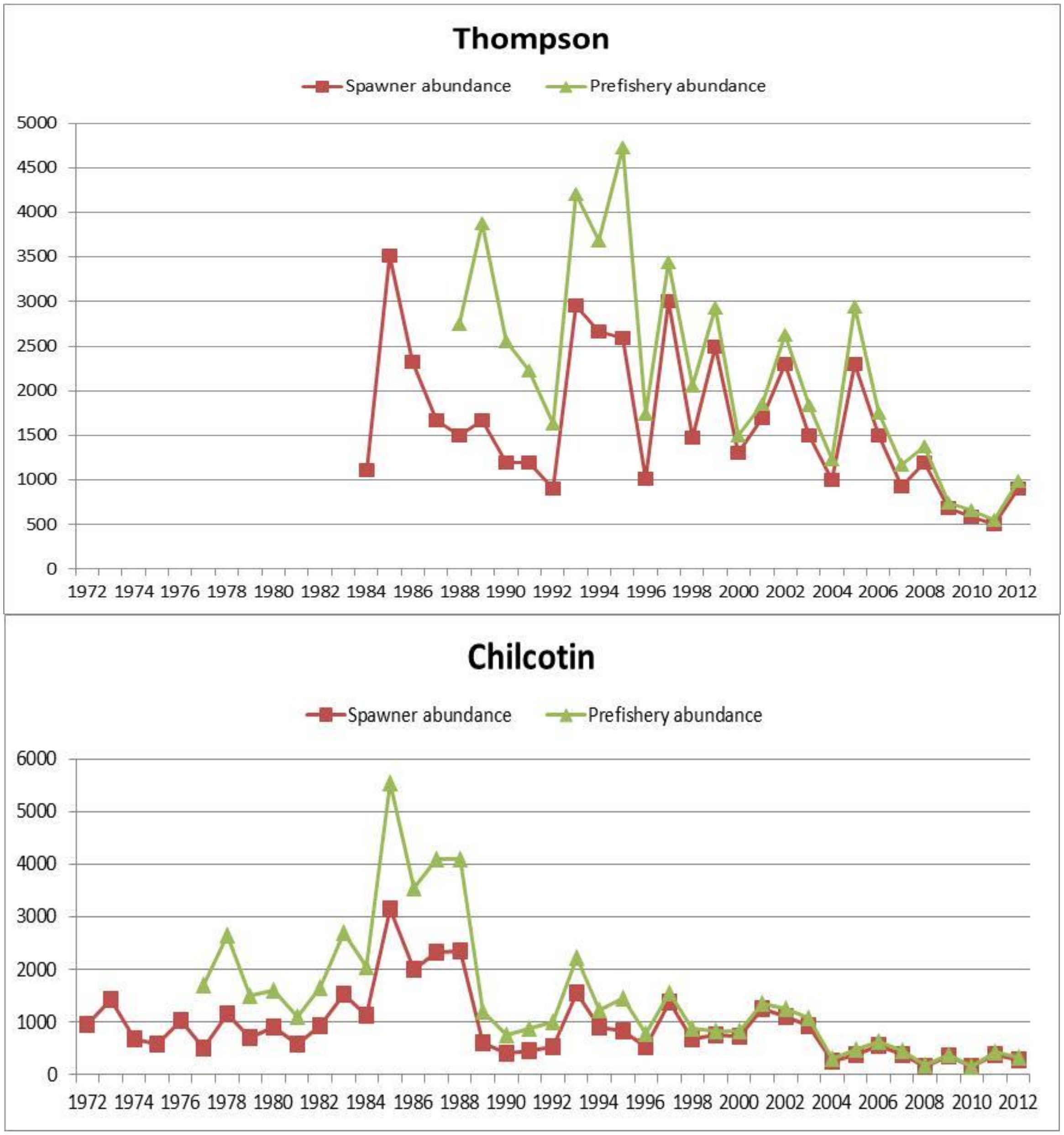


Figure 7. Thompson and Chilcotin River summer run steelhead abundance in British Columbia's Georgia Basin (Pollard and Beere 2012).

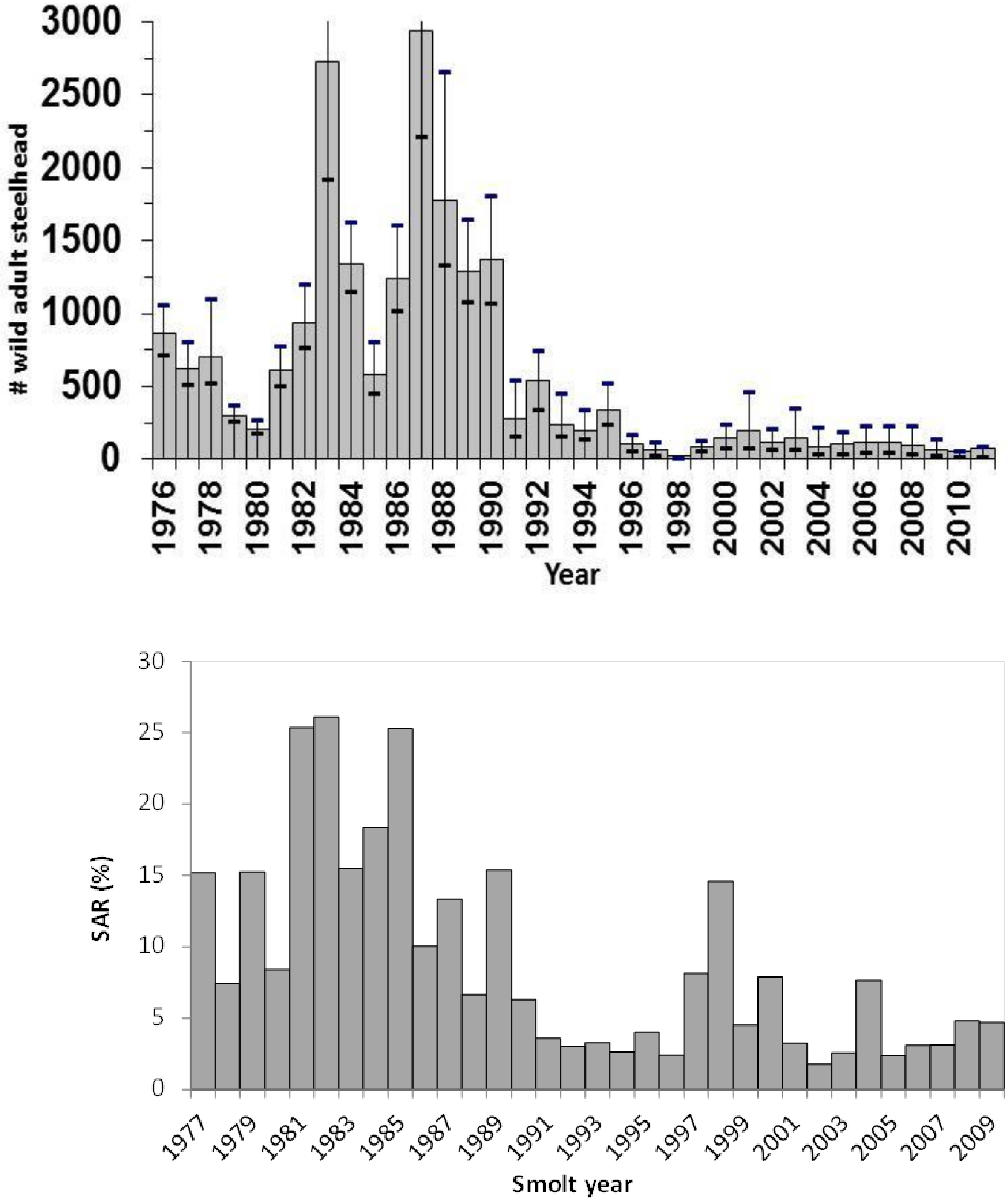


Figure 8. The abundance and marine (smolt-to-adult) survival of wild Keogh River winter steelhead (top panel = Pollard and Beere 2012, bottom panel = pers. comm. Tom Johnston, DFO, 2013).

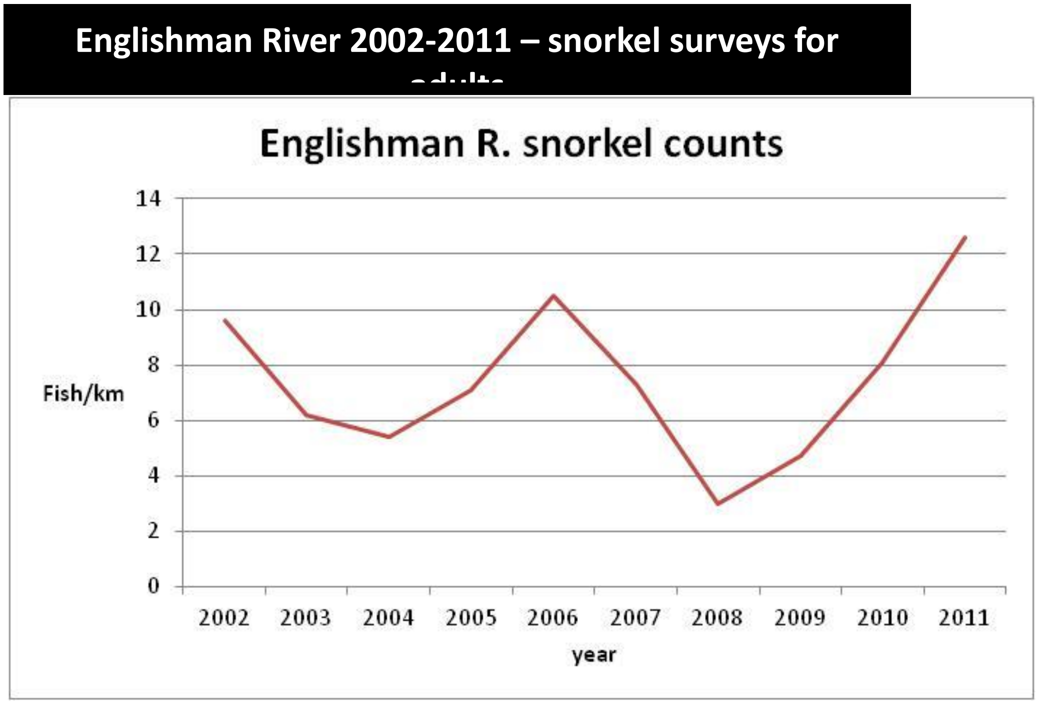


Figure 9. Density estimates for the Englishman river of the Georgia Basin (Pollard and Beere 2012).

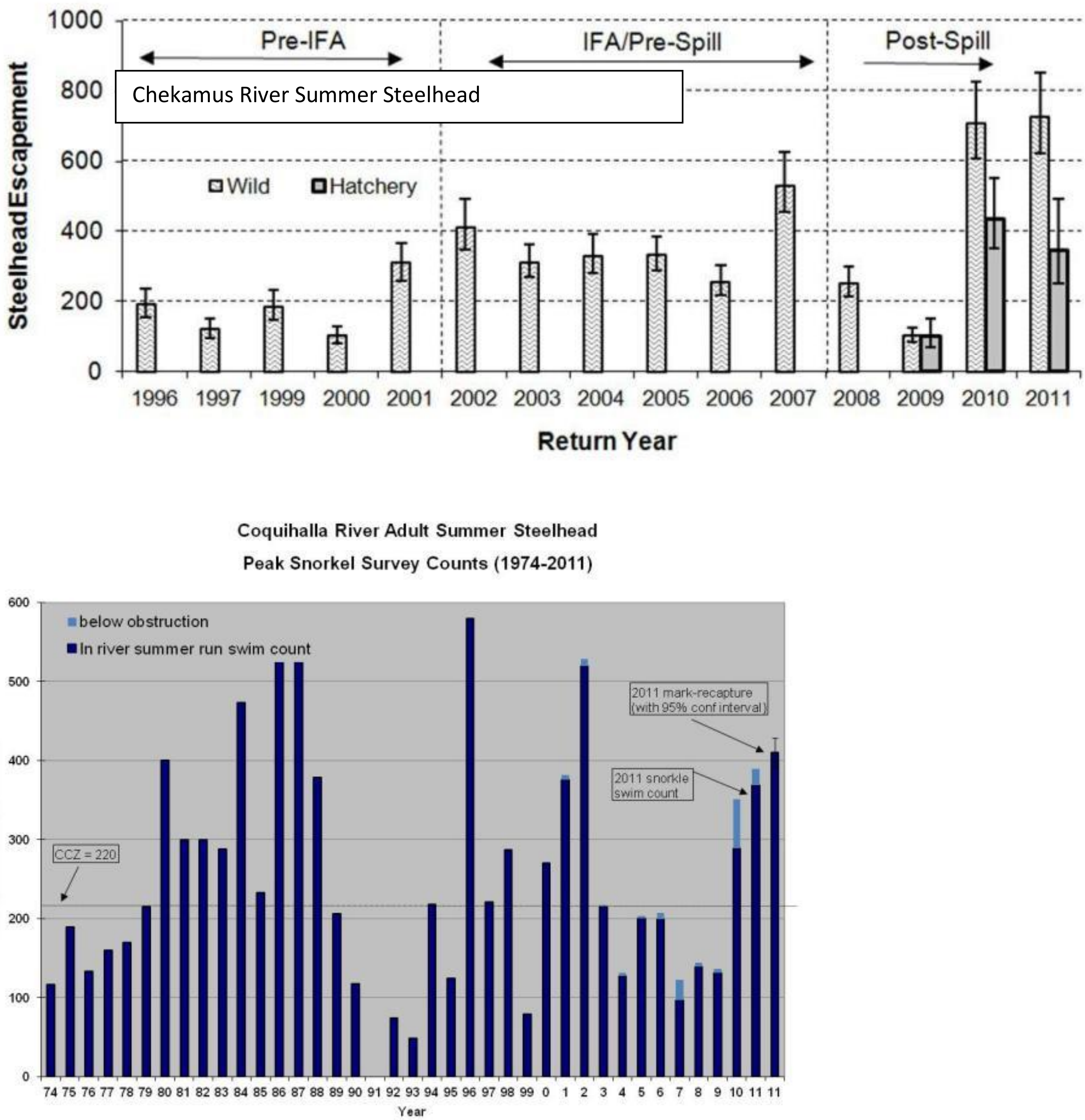


Figure 10. Adult summer steelhead abundance trends for the Chekamus and Coquihalia rivers in the Georgia Basin (Pollard and Beere 2012).

Where within Puget Sound is mortality occurring?

NAR = Tacoma Narrows
CPS = Central Puget Sound
ADM = Admiralty Inlet
HCB = Hood Canal Bridge
DP = Deception Pass

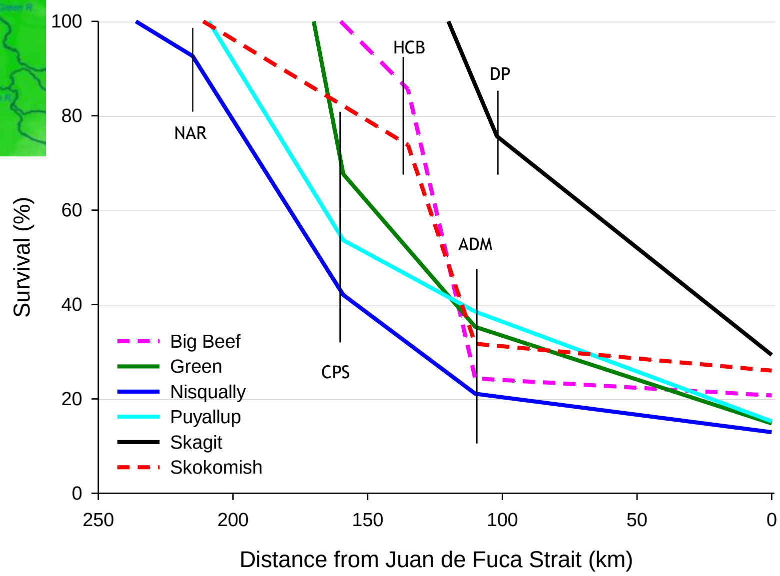


Figure 11. Early marine mortality of wild steelhead smolts (acoustic tagged populations) by migration segment, in Puget Sound, between 2006 and 2009 (Moore et al. NOAA, 2013, unpublished data).

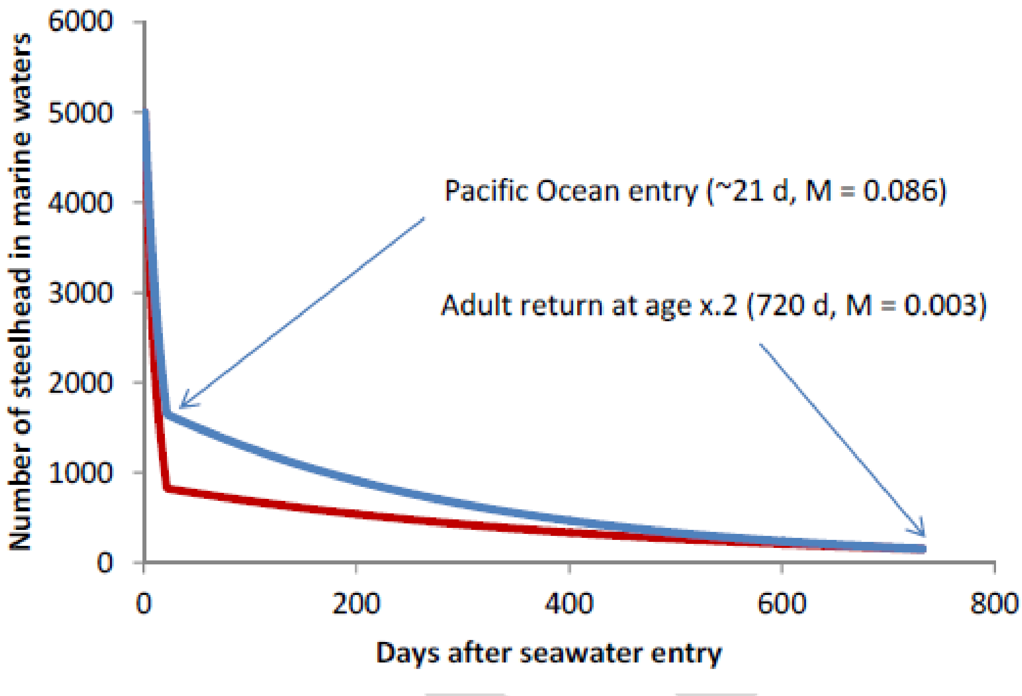


Figure 12. Survivorship curves reflect a hypothetical Puget Sound population of 5,000 smolts experiencing instantaneous daily mortality rates (M)estimated from acoustic telemetry data from river mouths to Pillar Point (western end of the Strait of Juan de Fuca) and based on a travel time of 21 days (Moore et al. 2010). Hypothetical mortality rates after 21 days were estimated by assuming SAR = 3% and steelhead returned after two ocean winters. Red line is based on actual telemetry survival estimates and the blue line reflects early marine survival estimates that are 2 times the empirically estimated rate (i.e., assuming we've underestimated survival to Pillar Point by 50%). Figure produced by B. Berejikian NOAA, 2012.

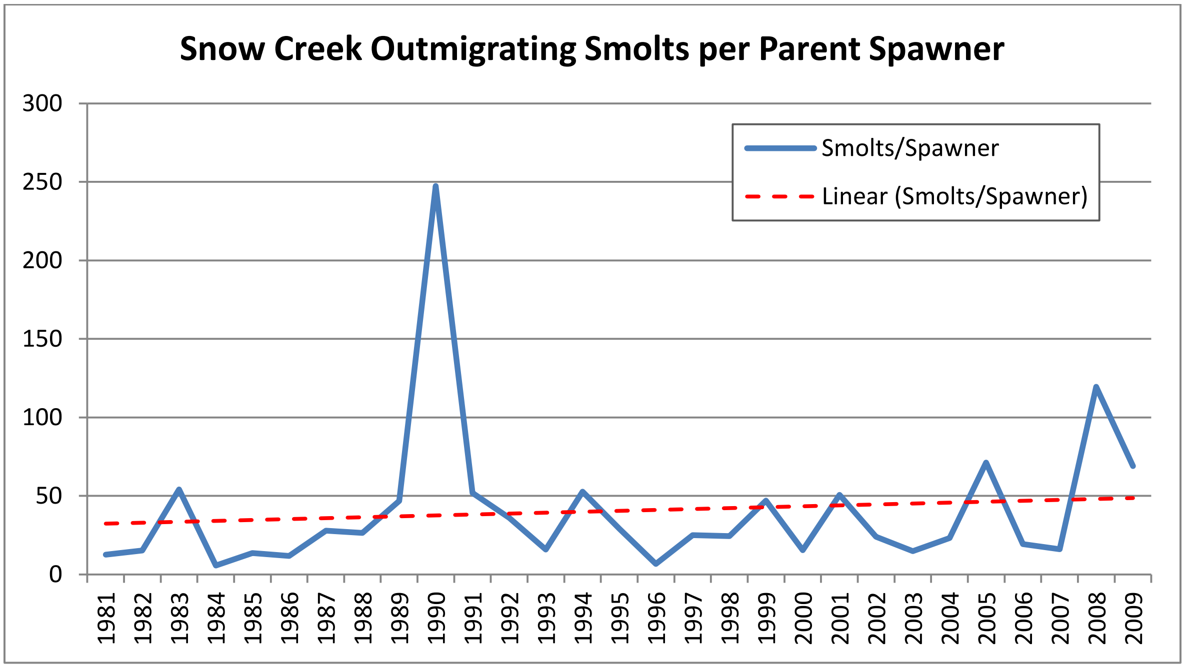


Figure 13. Snow Creek outmigrating smolts per parent spawner. Assumes outmigration at age 2, based upon mean age at outmigration (and representative of 85% of the population) for smolt years 1978-1990 (Johnson and Cooper 1992)[28]. Produced by Kemp, LLTK, using WDFW 2012 unpublished data.

[28] The smolt age structure data used are from 1978-1990. Updated age structure data to determine if any change has occurred in Snow Creek and similar evaluations of freshwater survival for inner Puget Sound steelhead, where the declines in SARs are more significant, would be beneficial.

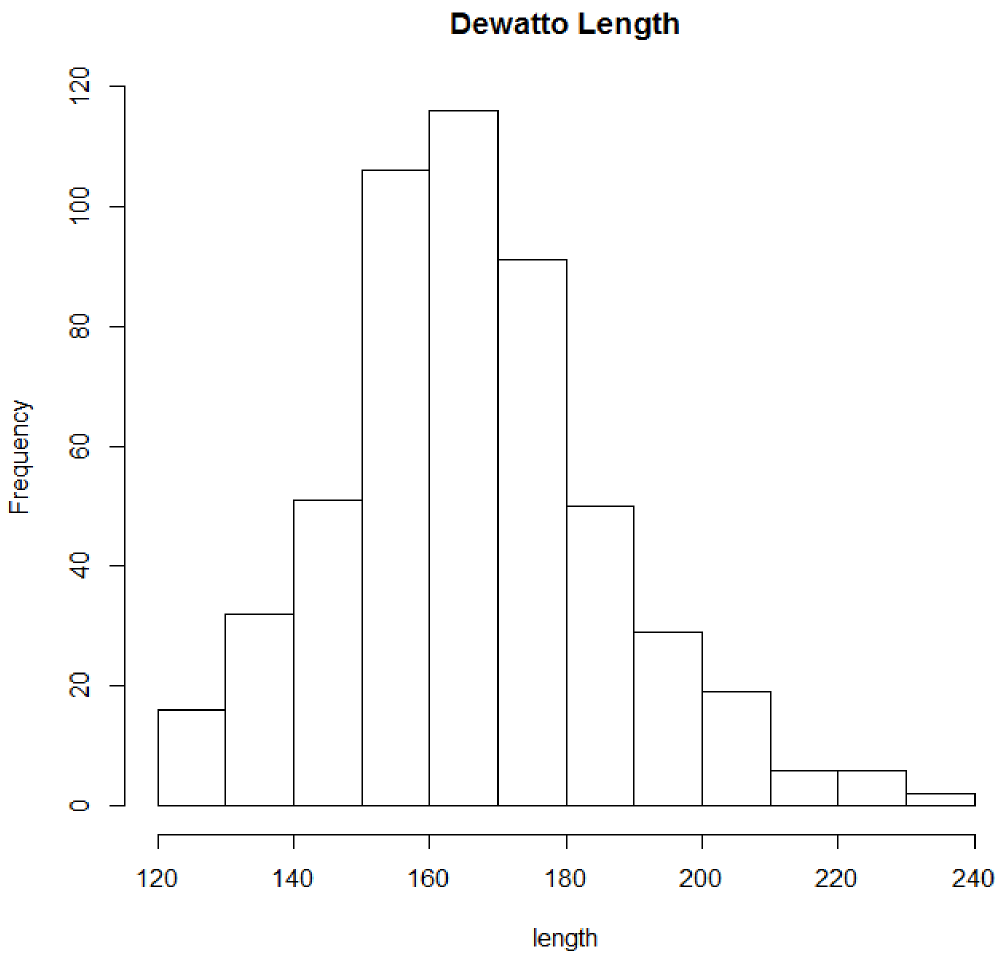


Figure 14. Size distribution of Dewatto winter steelhead outmigrants (Moore et al. 2013, unpublished data). Anecdotally, this is representative of typical size distribution throughout Puget Sound; however, more work is planned to evaluate size distribution.

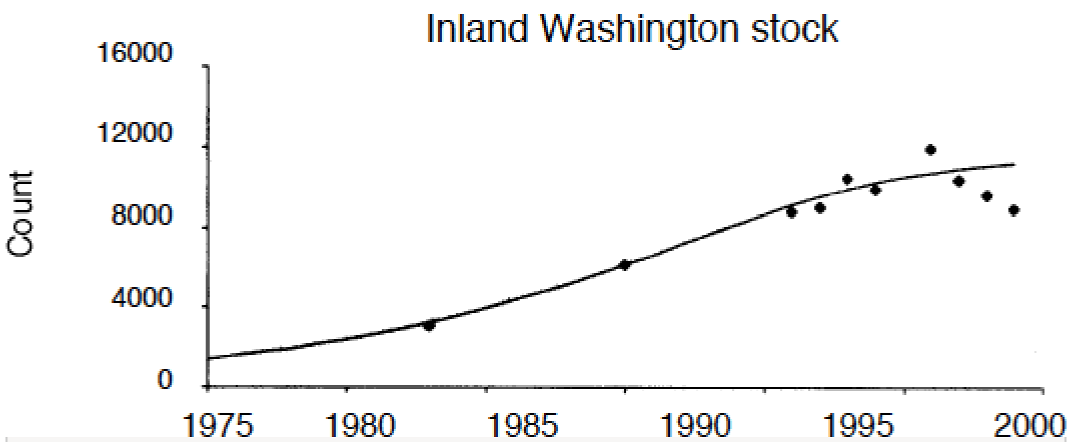


Figure 15. Generalized logistic growth curve of aerial counts of harbor seals in inland waters of Washington (includes the Strait of Juan de Fuca, East Bays, San Juan Islands, Hood Canal, and Puget Sound regions) (Jeffries et al. 2003).

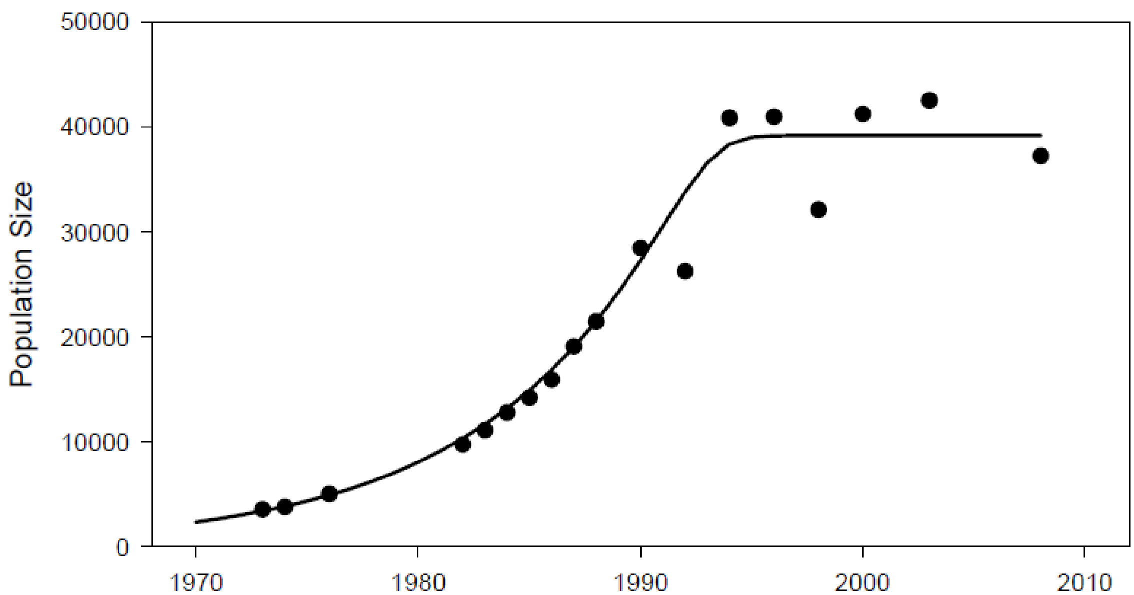


Figure 16. The increased abundance of harbor seals in the Strait of Georgia (Trites 2012 presentation). Similar to Puget Sound, the population has appeared to plateau; however, surveys have not been done recently.

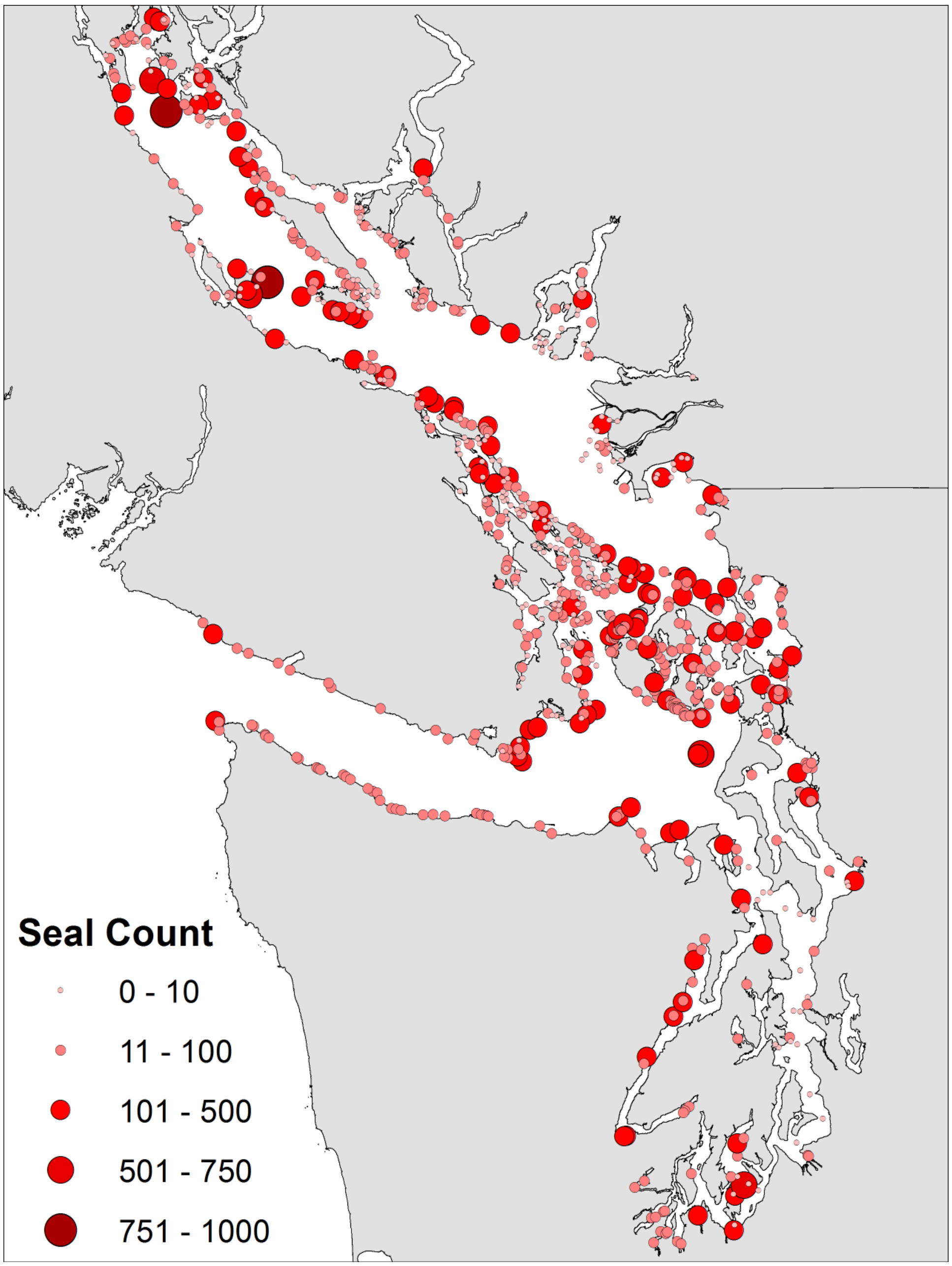


Figure 17. Harbor Seal distribution in the Salish Sea (Strait of Georgia and Puget Sound) (Trites 2012 presentation)

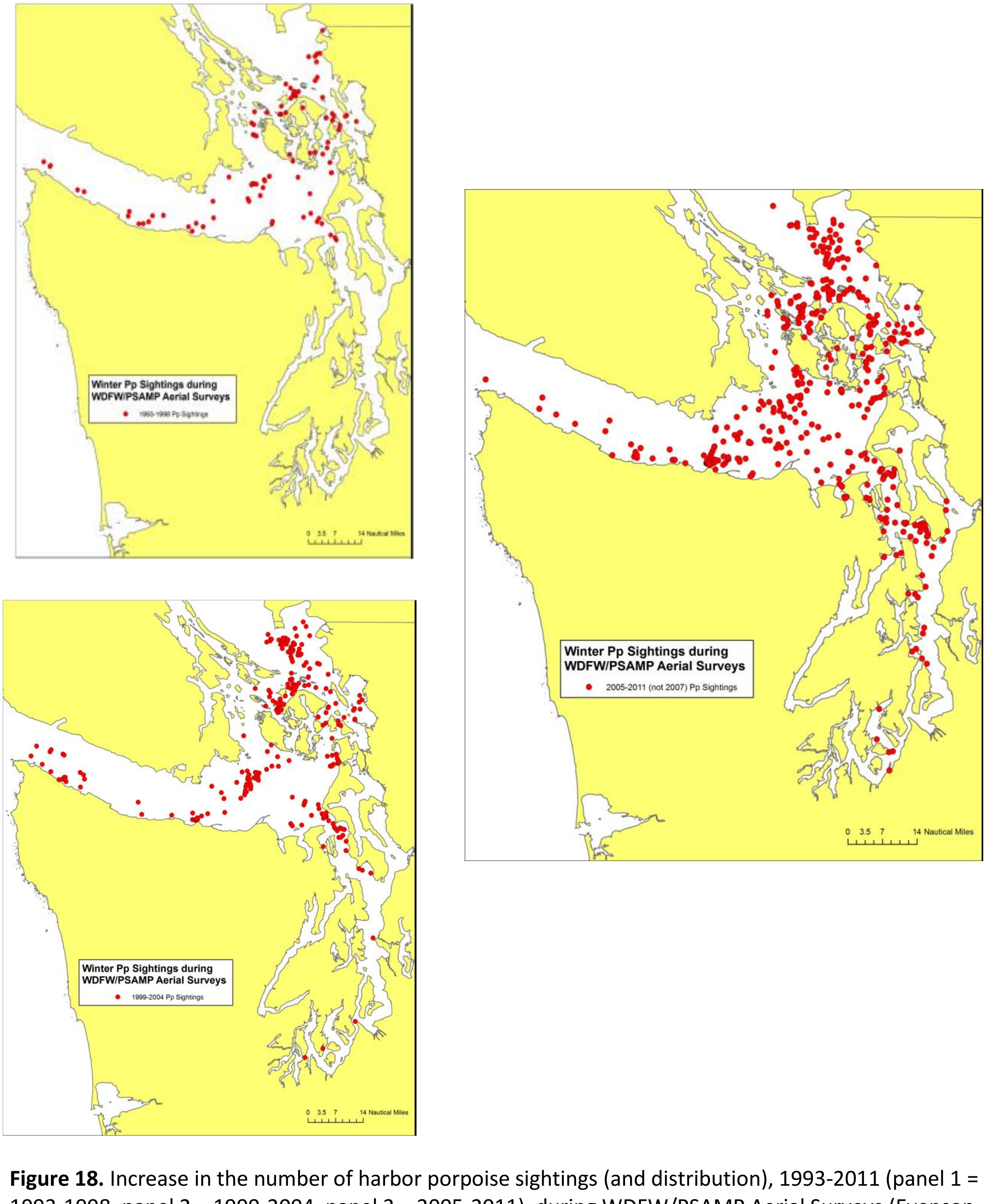


Figure 18. Increase in the number of harbor porpoise sightings (and distribution), 1993-2011 (panel 1 = 1993-1998, panel 2 = 1999-2004, panel 3 = 2005-2011), during WDFW/PSAMP Aerial Surveys (Evenson, WDFW, 2013, unpublished data).

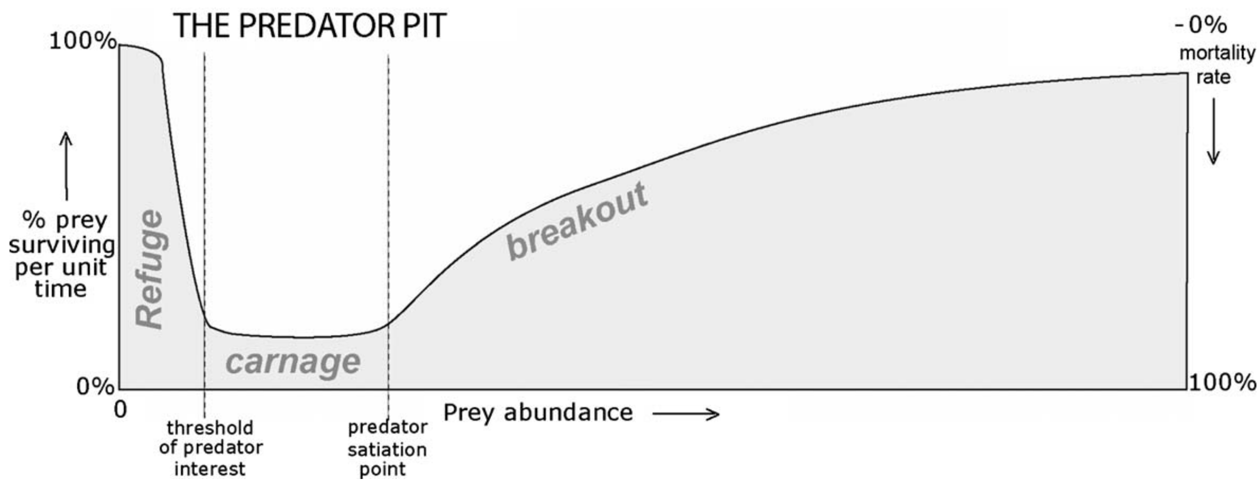
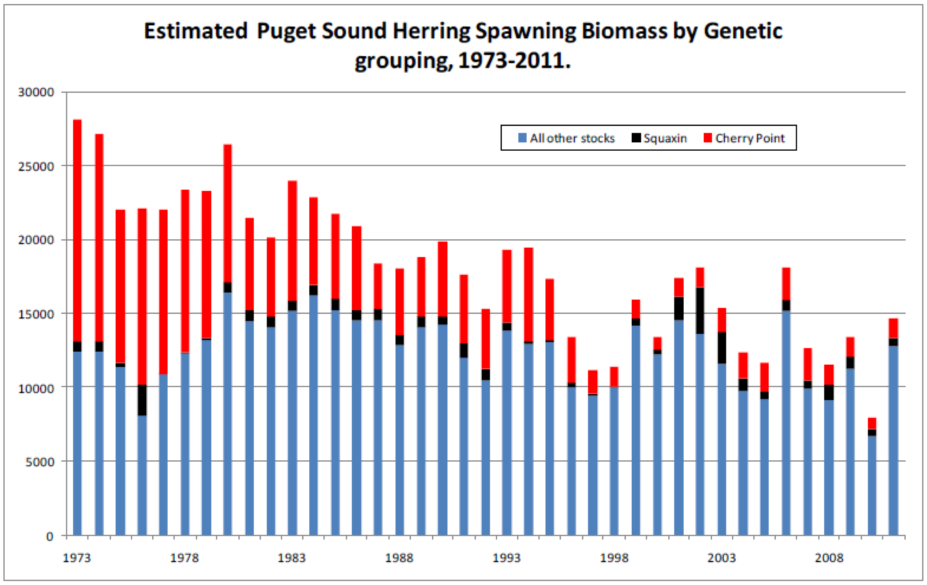


Figure 19. Diagram of the predator pit function. Survival rate (plotted on the left ordinate) increases upwards. Mortality rate (plotted on the right ordinate) increases downwards (Bakun 2006).



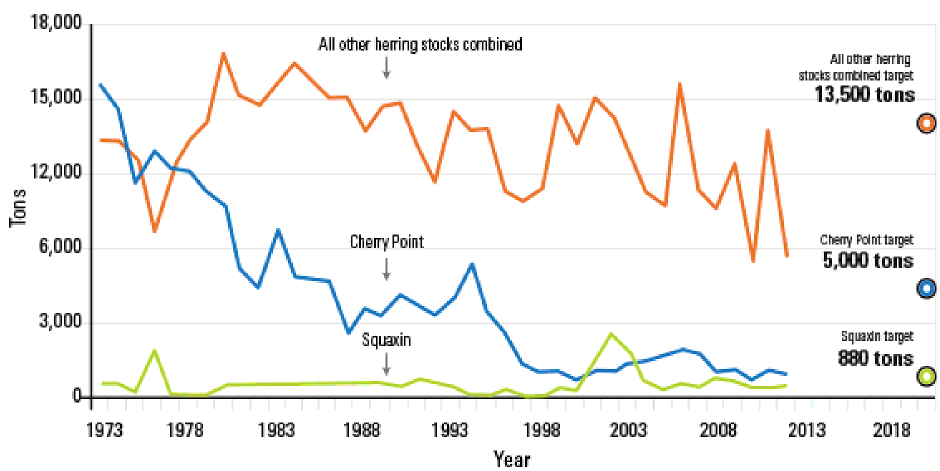


Figure 1. Annual estimates of Puget Sound herring spawning biomass, by genetic grouping with associated targets (1973 to 2012).
Source: Washington Department of Fish and Wildlife, Fish Program

Figure 20. Annual estimates of Puget Sound herring spawning biomass 1973-2011. The overall Puget Sound herring population has declined; however this is largely the result of the significant decline in the Cherry Point spawning population, which historically represented half of the total spawning biomass in Puget Sound. Top Figure from the Pacific Herring Information Summary (WDFW 2012), bottom Figure from Puget Sound Vital Signs page, http://www.psp.wa.gov/vitalsigns/pacific_herring.php#! .

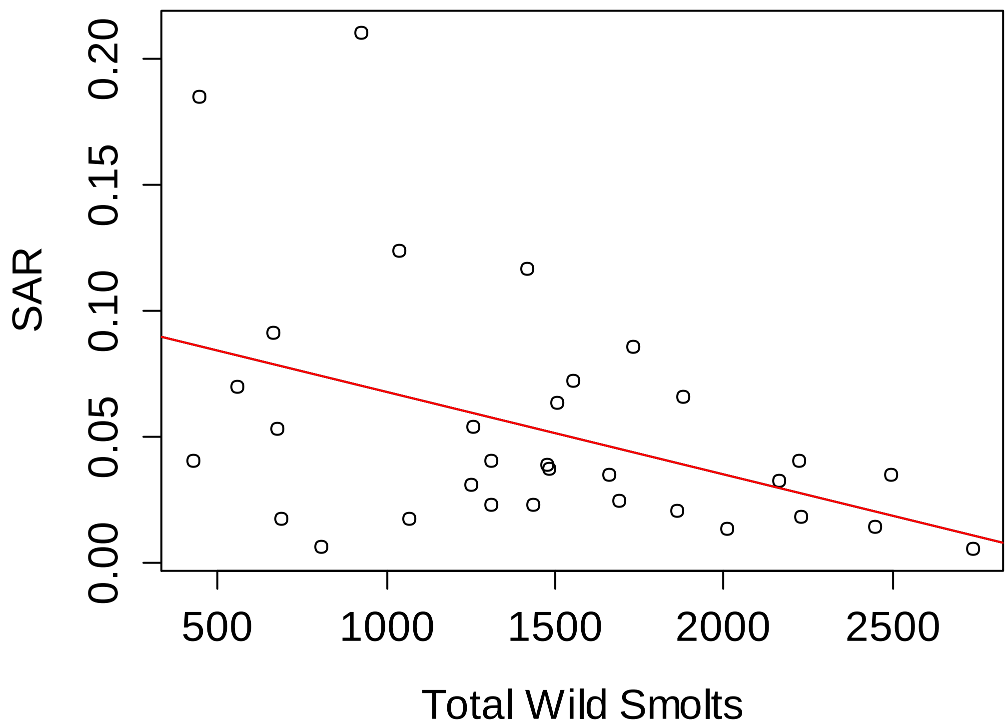


Figure 21. Snow Creek juvenile outmigrant abundance relative to overall survival. A linear regression on untransformed data indicated a negative relationship between smolt-to-adult survival and total wild smolts (adj R-squared=0.15, p=0.015). Produced by Kemp, LLTK, using WDFW 2012 unpublished data.

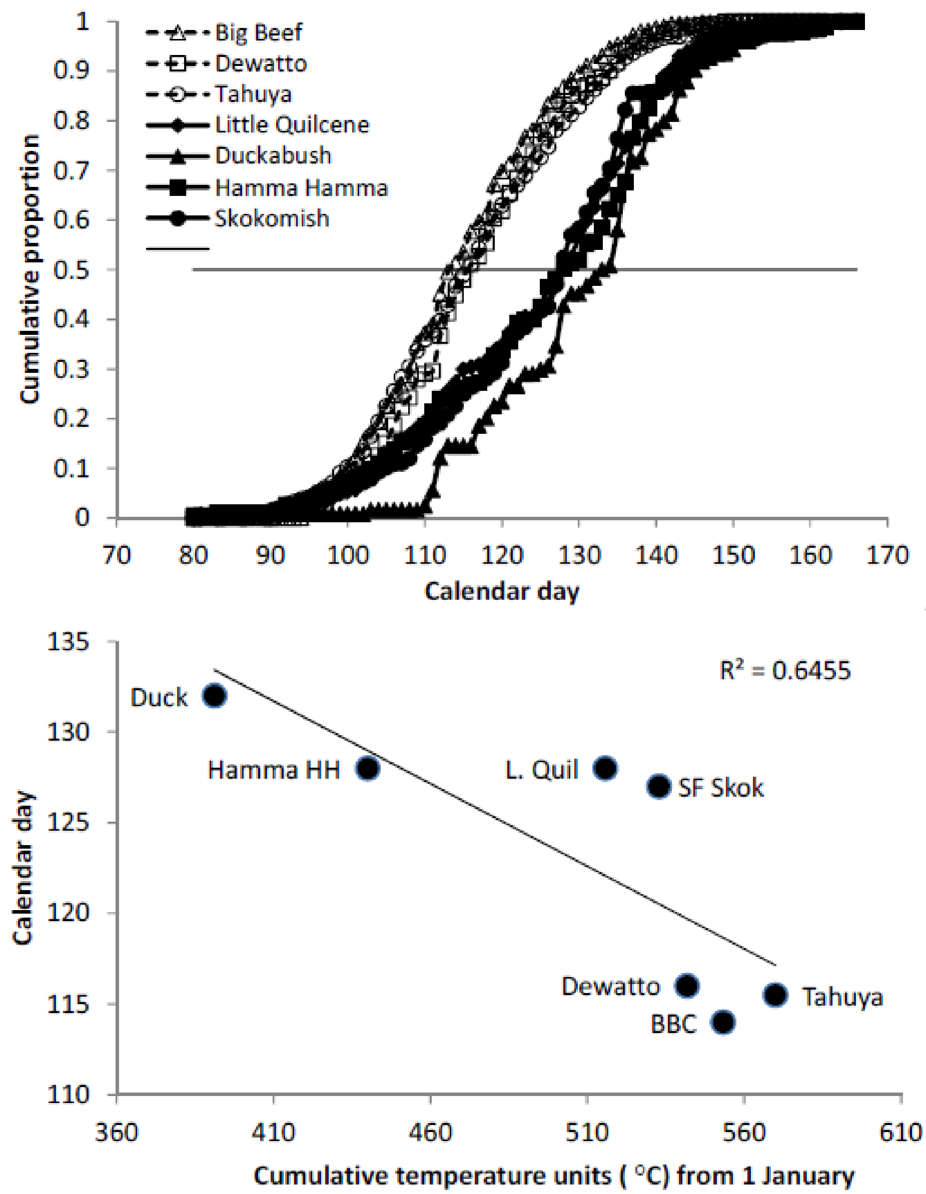


Figure 22. Cumulative proportion of steelhead smolt outmigrants from Kitsap Peninsula (open symbols) and Olympic Peninsula (black symbols) populations (top panel). Cumulative temperature units (degrees °C x days) plotted against mean date of outmigration (bottom panel). Data represent average of two (Little Quilcene), three (Duckabush) or four years of outmigration data (all other populations). Temperatures are annual averages of average monthly temperatures.

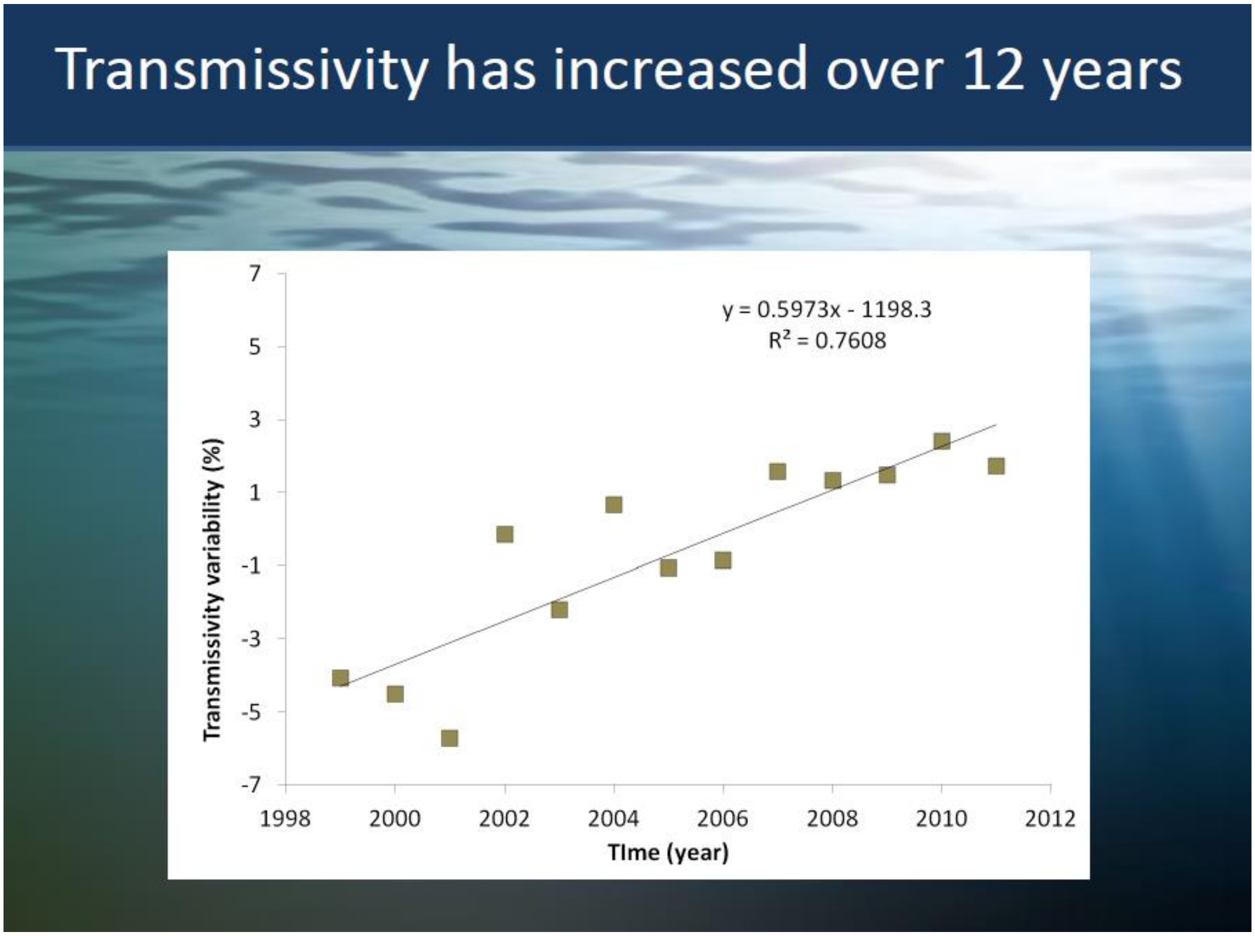


Figure 23. Increase in water clarity in Puget Sound, 1998-2011 (Krembs 2013).

Appendix 1: Hatchery stocks included and brood years available for SAR analysis

Winter-run steelhead
Puget Sound:
From Scott and Gill 2008--
Skagit 1975-1991 (otherwise Skagit stock not included because hatchery fish spawning in the wild were not counted)
Elwha 1980-1991
Puyallup 1976-1992
WDFW hatchery program data--
Nooksack 1993-2009
Stillaguamish 1992-2009
Snohomish 1993-2009
Green 1993-2009
Puyallup 1994-2008
South Sound 1992-2004 (outplanted population but included because only south Sound stock)
Hood Canal 1992-2002
Dungeness 1992-2009

Lower Columbia:
From Scott and Gill 2008--
Elochoman 1984-1992
Kalama 1974-1992
Washougal 1984-1992
WDFW hatchery program data--
Cowlitz 1992-2009
Elochoman 1993-2009
Grays River 1995-2009
Kalama River 1993-2009
Lewis River 1992-2009
Washougal River 1993-2009

Coast:
From Scott and Gill 2008--
Quillayute 1975-1991
Quinault 1975-1991
Humptulips 1991
Chehalis 1979-1991
WDFW hatchery program data--
Bogachiel 1993-2009
Humptulips 1993-2009
Naselle 1993-2009
Satsop 1992-2009
Skookumchuck 1992-2009
Willapa 1993-2009
Wynoochee 1992-2009

Summer-run steelhead
Puget Sound:
No stocks in Scott and Gill 2008
WDFW hatchery program data--
Snohomish 1993-2008
Green 1993-2008

Lower Columbia:
From Scott and Gill 2008--
Kalama 1973-1992
Washougal 1976, 1983-1991
WDFW hatchery program data--
Cowlitz 1993-2008
Kalama 1993-2008
Lewis 1993-2008
Washougal 1992-2008

Lower Columbia:
No stocks in Scott and Gill 2008
WDFW hatchery program data--
Humptulips 1993-2008
Quilayute 1993-2008
Wynoochee 1993-2008

* **Scott and Gill 2008** data used when updated data were not available from WDFW hatchery program records. Some years are missing data in these time series

Appendix 2: Fish health recommendations to the workgroup[29]

July 22, 2013

Fish Health Evaluation Participants:

Bruce Stewart (NWIFC), John Kerwin (WDFW), Andy Goodwin (USWFS), Joy Evered (USFWS), Paul Hershberger (USGS), Linda Rhodes (NOAA), and Kym Jacobson (NOAA)

Goals:

- To develop a prioritized list of fish pathogens with the potential to influence the early marine survival of steelhead populations in Puget Sound.
- Specify the sampling environments for each pathogen screen including: (1) In-river freshwater sampling locations including smolt traps and hatchery releases, (2) Downriver and estuary locations, and (3) Marine locations.
- Identify appropriate test methods and target tissues for detection of each pathogen.

Discussion Points:

- The fish health group reviewed possible diseases/pathogens that may contribute to the zoographic patterns that are currently observed in Puget Sound steelhead populations including:
    - Relatively rapid mortality occurring in affected steelhead populations shortly after they enter seawater (1-2 weeks).
    - Fish originating from South Sound and Hood Canal regions are being affected more than fish originating from North Sound and Coast.
    - Inter-species mortality patterns also occur in coho and Chinook stocks in South Sound.
- The group broke down sources of pathogens into the following two groups and divided them into high, moderate, and low priority categories:
    - Pathogens that the steelhead are acquiring in freshwater (hatchery or natural systems).
    - Pathogens that the steelhead are acquiring/being exposed to in saltwater.
- Test methods considered included culture, molecular methods (PCR), histology, and a fish health exam with skin scrapes and wet mount observations of tissues.

[29] This section was prepared by Bruce Stewart, NWIFC. The fish health evaluation participants are listed at the beginning of this section.

- A table is provided at the end of this appendix summarizing the group's conclusions.

Recommended approach:

1. **<u>Highest priority pathogen category</u>**: Screening for *Nanophyetus salmincola* in wild and cultured steelhead stocks.
   - Rationale: From a pathogen perspective, *Nanophyetus* represents the strongest candidate for contributing to the observed mortality patterns in Puget Sound steelhead:

     1) The 3 salmonid species experiencing the greatest mortalities (steelhead, coho, and Chinook) are highly susceptible to *Nanophyetus*.

     2) Within the Salish Sea, *Nanophyetus* occurs in high prevalence and intensity among salmonids in the watersheds with the lowest returns (south Sound and Hood Canal).

     3) High infection intensity among freshwater outmigrants could result in rapid mortality shortly after seawater entry (Jacobson et al. 2008) especially in consideration of decreased swimming performance (Butler and Millemann 1971) accompanied with a predisposition of diseased individuals to predation.
   - Recommended screening to include the following:
     - Conduct an expansive surveillance of *Nanophyetus* among hatchery and wild fish in all three sampling environments (in-river freshwater collection sites such as smolt traps and pre-release screening of hatchery steelhead stocks), downriver and estuary collection sites, and marine sites.
     - Conduct paired *Nanophyetus* samples to accompany the proposed transplant study between two South Sound watersheds.
   - Test method/Tissues Collected:
     - Primary screening test method would be enumerating the number of cysts in the posterior 1/3 of kidney using light microscopy.
     - Secondary test methods: In addition, we recommend collecting and fixing tissues from all/or a subset of sampled fish for histology examination (kidney, heart, brain, liver, and gill). These samples would be archived and available for future testing to determine histopatholgical caused by the infection.
   - Expected outcome: By following the infection prevalence and intensity of *Nanophyetus* in cohorts of outmigrating steelhead, this approach will indicate whether infected individuals experience mortality during outmigration and early seawater entry. If the results support the hypothesis that infected individuals experience elevated mortality, then additional controlled studies will be recommended to address cause-and-effect.

2. **<u>Moderate priority pathogen category</u>**: Efforts to be placed on screening for a second tier of pathogens that are known to be present in Puget Sound and have the potential to cause mortality in steelhead as well as other salmon. The fish health workgroup put the risk as moderate that these pathogens were involved either as a direct cause or contributing cause to the reported loss in steelhead.

- Moderate priority pathogens include:
    - Bacteria: *Listonella (Vibrio) anguillarum, L. ordalli,, Aeromonas salmonicida, Tenacibaculum (Flexibacter) maritimum, Flavobacterium psychrophilum*,
    - HABs: *Chaetoceros spp.*
    - Rickettsia: *Piscirickettsia salmonis*
- Sampling locations: All three locations including freshwater, estuary, and marine locations.
- Test methods: Refer to attached spreadsheet for recommended primary screening assays. In general, we are recommending that regardless of the primary screening assay used, that tissues should also be collected from all sampled fish and either fixed for histology or PCR testing at a later date if warranted.
    - Histology: Tissues to fix include kidney, heart, liver, brain, and gill.
    - PCR: Tissues to store in RNA later include kidney, liver, and gill.
- Expected outcome: This approach will indicate the prevalence and intensity of certain marine pathogens in steelhead. Due to limited sampling opportunities among the same cohorts at multiple freshwater and seawater sites, a longitudinal assessment for survival effects may not be possible.
- References

Butler, J.A., R.E. Millemann. 1971. Effect of the “salmon poisoning” trematode, *Nanophyetus salmonicola*, on the swimming ability of juvenile salmonid fishes. Journal of Parasitology 57: 860-865.

Jacobson, K.C., D. Teel, D.M. VanDoornik, E. Casillas. 2008. Parasite-associated mortality of juvenile pacific salmon caused by the trematode *Nanophyetus salmonicola* during early marine residence. Marine Ecology Progress Series 354: 235-244.

3. **Low priority pathogen category**: Efforts to be placed on screening for a much more expansive list of pathogens that have been associated with steelhead losses either in the region of concern or in other areas. The group felt that these pathogens had a low likelihood of being the primary cause of loss being reported but could be a contributing factor to the problem:

- Low level priority pathogens include:
    - Bacteria: *Renibacterium salmoninarum.*
    - Viruses: Culturable and emerging viruses
    - Parasites/Fungi: sea lice (*Lepeopthirius, Caligus, Argulus spp*.), *Parvicapsula minibicorbis, Loma salmonae, Nucleospora salmonis, Tetracapsuloides bryosalmonae, Dermocystidium spp., Cryptocaryon spp., Sphaerothecum destruens (Rosette Agent),* and *Ichthyophonus*.
- Sampling locations: All three environments including in-river freshwater sites, estuary, and marine collection sites.
- Test methods: Refer to attached spreadsheet for recommended primary screening assays. We are recommending that regardless of the primary screening assay used, that tissues should also

be collected from all sampled fish and fixed appropriately for histology or PCR testing at a later date if warranted.

- Histology: Tissues to fix include kidney, heart, liver, brain, and gill.
- PCR: Tissues to store in RNAlater include kidney, liver, and gill.

- Expected outcome: This approach will indicate the prevalence and intensity of certain marine pathogens in steelhead. Due to limited sampling opportunities among the same cohorts at multiple freshwater and seawater sites, a longitudinal assessment for survival effects may not be possible.

Table 1. List of priority pathogens for evaluation in juvenile steelhead salmon in Puget Sound. Sample collection methods will be tailored to the test methods in bold text.

Priority	Pathogen	Class	Primary Test Method	Alternate Test Method	Tissues	Sampling Location	Comments
High	*Nanophyetus salmincola*	Parasite (digentic	**Microscopic**	**Histology**[1]	Posterior Kidney; Other organs by histology	FW/Marine	High prevalence and severity in region
Medium	*Listonella (Vibrio) anguillarum, L. ordalii*	Bacteria	Culture	**PCR**[2]	Kidney	Marine	Pathogens that are either associated with steelhead losses in WA or are known causes of loss in Atlantic salmon marine pen culture in WA. These pathogens have a moderate risk of being associated with the steelhead loss.
Medium	*Aeromonas salmonicida*	Bacteria	Culture	**PCR**	Kidney	FW/marine	
Medium	*Tenacibaculum (Flexibacter)*	Bacteria	Culture	**PCR**	Kidney	Marine	
Medium	*Flavobacterium psychrophilum*	Bacteria	Culture	**PCR**	Kidney	FW/marine	
Medium	*Piscirickettsia salmonis*	Rickettsia	**PCR**	**Histology**	Kidney	Marine	
Medium	*HABs (Chaetoceros spp.)*	HABs	Gill exam (wet mount or histo)	**Histology**	Gills	Marine	
Medium	*Sea Lice*	Copepod parasites	**Visual exam**	None	External and gills	Marine	
Low	*Parvicapsula minibicornis*	Parasite (myxosporean)	**PCR**	**Histology**	Kidney, gill	FW/Marine	Pathogens that do not fit the mortality pattern described for steelhead in the affected regions but could be a contributing factor.
Low	*Loma salmonae*	Parasite (microsporidian)	**PCR**	**Histology**	Kidney, gill	FW/Marine	
Low	*Nucleospora salmonis*	Parasite (microsporidian)	**PCR**	**Histology**	Kidney, gill	FW/Marine	
Low	*Tetracapsuloides bryosalmonae*	Parasite (myxosporean)	**PCR**	**Histology**	Kidney	FW/Marine	
Low	*Dermocystidium*	Parasite	**PCR**	**Histology**	Gills	FWMarine	
Low	*Cryptocaryon*	Parasite	Microscopic exam	**PCR**	Skin/gills	Marine	
Low	*Sphaerothecum destruens (Rosette Agent)*	Parasite	**Histology**	**PCR**	Kidney	Marine	
Low	*Ichthyophonus spp.*	Fungus	Culture	**PCR**	Heart	Marine	
Low	Culturable viruses	Virus	Cell culture	**PCR**	Kidney/spleen	FW/Marine	
Low	Emerging viruses	Virus	**PCR**	None	Kidney/gill/heart	FW/Marine	
Low	*R. salmoninarum*	Bacteria	**PCR**	IFAT	Anterior kidney	FW/Marine	

[1]Histology samples to include gill, heart, kidney, brain and liver fixed in an appropriate fixative (10% NBF, Davidsons)

[2]PCR samples to include kidney, liver, and gill stored in RNAlater. Posterior kidney can be used for both microscopic exam and PCR.

Appendix 3: Complete descriptions of research components

1) Study 1: Puget Sound-wide analysis of steelhead acoustic telemetry data

Principal Investigator: Megan Moore (NOAA)

This study and its preliminary results are described in section 1.2 of this report. The analysis will be completed and manuscript produced by early 2015.

Studies 2-4: Assessing smolt-to-adult return (SAR) data to reveal underlying patterns of early marine survival in Puget Sound

For these studies, we will collect hatchery and wild steelhead SAR data from Puget Sound, the Washington coast, and the lower Columbia River, assess these data for trends over time, and relate the SARs and other metrics of marine mortality to smolt characteristics, population life-history data, environmental data, and the buffer prey effect. This will help elucidate the spatial and temporal scales of the steelhead marine survival declines and what individual fish, population-scale, and environmental factors are related to the decline. We predict that these three studies will be completed by spring 2015.

2) Study 2: Assessing existing SAR data for trends

Principal Investigator: Neala Kendall (WDFW)

2.1 Justification

Smolt-to-adult return (SARs) values, especially for coastal steelhead populations representing the proportion of fish that survive from entry into marine waters to maturation, best represent marine survival rates. Tracking trends in these values is vital for determining the spatial and temporal scales of the problem. These data represent some of the most basic information needed in the steelhead marine survival project and can be used in a number of additional analyses.

2.2 Background and hypotheses

In this study we will assess the declining trends in marine survival of both hatchery and wild steelhead in Puget Sound and other neighboring regions, estimated using cohort reconstructions to calculate SARs.

Study 2 addresses the following questions:

1. **Is marine survival significantly different for hatchery and wild populations in Puget Sound relative to Washington Coast and Lower Columbia?**

   We will compare SAR values in Puget Sound with those in neighboring regions. We hypothesize that SAR values have declined more for Puget Sound steelhead populations than for populations in neighboring regions given the steeper declines in abundance for Puget Sound populations.

2. **Do we see differences in SAR trends among Puget Sound populations? And if so, where, when, and to what degree have they varied in space or time?**

   We will evaluate variability of SAR trends among Puget Sound populations to understand if and how marine survival differs for stocks entering the Sound within different sub-basins. These questions can help narrow down the scale and possible sources of mortality and thereby identify the most likely causes of increased mortality in Puget Sound.

3. **How does marine survival differ between hatchery and natural origin/wild steelhead in Puget Sound?**

   We will assess whether marine survival differs between hatchery stocks and naturally spawning populations. Wild steelhead can spend many years in freshwater and thus their SARs can be influenced by their state and thus freshwater conditions, whereas hatchery stocks generally have short freshwater and estuarine residency and thus most of the SAR can be inferred to be influenced by marine sources. Finally, this population-specific assessment will provide the foundation for correlative analyses with factors potentially affecting survival.

4. **Does marine survival more strongly predict adult returns than freshwater survival?**

   Trends in survival and variation in total survival for wild steelhead will be partitioned between the freshwater and marine environment, and the correlation between freshwater and marine survival rates and resulting adult recruits will be analyzed. This exercise will be limited by the datasets available to perform this analysis (e.g., Snow Creek, Keogh River).

2.3 Materials and methods

We will first estimate SARs for state, tribal, and federal steelhead-producing hatcheries in Puget Sound, Strait of Georgia, Washington coast, and the lower Columbia River. Data needed include hatchery release, harvest, and hatchery return counts for fish of a given release year. Second, we will estimate SARs for wild populations in these regions using data from juvenile traps that have collected steelhead smolts and escapement data of adults (along with the proportion of hatchery fish) of a given outmigration year. We will use juvenile and adult fish abundance, adult age structure, and proportion wild fish in the SAR estimates.

2.4 Specific statistical methods to evaluate the SAR trends include regressions (to test for changes in slope over time), time series methods (to test for the presence of variations and patterns over time), and correlations (to examine relationships between SAR trends from different regions). Implications and next steps

This study is designed to answer questions about spatial and temporal patterns of marine survival of steelhead populations. In further analyses described in Studies 3 and 4, these SAR data will be statistically analyzed and correlated with various steelhead individual and population characteristics along with Puget Sound environmental data, in part to account for variability in the estimates.

2.5 Timeline

We have collected SAR data for wild steelhead from 3 Puget Sound DPS populations, 6 Washington coast populations (encompassing the Olympic Peninsula and Southwest Washington DPSs), and 4 lower Columbia River DPS populations over a range of outmigration years between 1977 and present. Hatchery steelhead SAR data have been collected for 3 summer-run populations and 11 winter-run populations in Puget Sound, 3 summer-run populations and 8 winter-run populations on the Washington coast, and 5 summer-run populations and 7 winter-run populations in the lower Columbia River for ocean entry years 1975-present. Analyses of the SAR trends are ongoing.

3) Study 3: Evaluating smolt characteristics and population life-history data vs. SARs

Principal Investigator: Neala Kendall (WDFW)

3.1 Justification

The portfolio effect suggests that diversification minimizes the volatility of the investment, or, in this case, steelhead abundance and productivity (Lehman and Tilman 2000, Koellner and Schmitz 2006, Schindler et al. 2010). This effect describes why more diverse systems are more stable. By understanding individual fish and population traits that are correlated with SARs, and specifically related to higher values, we can identify characteristics important to maintaining healthy populations. Factors that influence the balance of the life history portfolios across and within populations are considered crucial to recovery (Carlson and Satterthwaite 2011, McPhee et al. in press).

3.2 Background and hypotheses

We will analyze existing data on wild and hatchery summer- and winter-run steelhead individual and population characteristics and relate these values to SAR trends over time. Ideally, this effort would also include developing new data collection stations for additional populations into the future. The goal of this work would be to evaluate whether a loss of population life-history diversity is associated with declining SAR rates and examine hypotheses concerning spatial variation in mortality, size-selective mortality, match-mismatch, and life history variation.

Study 3 addresses the following questions:

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

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

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

   We hypothesize that SAR trends are correlated with population life-history diversity and that populations that have increased introgression and declining diversity will have steeper SAR declines and lower current SAR values.

3.3 Materials and methods

SAR data for Puget Sound (and coastal and lower Columbia River populations, where relevant) will be statistically analyzed and correlated with changes in hatchery and wild smolt abundance to determine whether decreases or increases in juvenile abundance may be affecting predator-prey interactions (high abundance resulting in buffering or low abundance resulting in predators ignoring steelhead) or whether high abundances could be correlated with density-dependent effects. This evaluation will be performed at the watershed, sub-region (south Puget Sound, central Puget Sound, north Puget Sound, Hood Canal, Strait of Juan de Fuca), and Puget Sound region level.

The SAR data for Puget Sound (and coastal and lower Columbia River populations, where relevant) will be statistically analyzed and correlated with individual and population characteristics to account for variability in the estimates and help determine whether certain characteristics are contributing uniquely to mortality *(or are uniquely affected by the environment)* in Puget Sound.

The following characteristics will be included in this analysis: summer vs. winter run; hatchery broodstock type (e.g., Chambers, Skamania, native); broodstock management approach used for hatchery programs (integrated vs. segregated); hatchery/wild composition and introgression in natural-origin populations; hatchery and wild smolt age, size, and condition factor; wild smolt outmigration timing; stream temperature and flow patterns; hatchery smolt release timing; kelt survival; wild and hatchery adult fish return migration timing, age and size; effects of mark-selective fisheries; and origin and migration patterns (from natal stream through Puget Sound).

We will also examine trends in these characteristics over time and also among fish from the different regions (Puget Sound vs. coast vs. lower Columbia). Of note, shifting climate conditions have the potential to interact with these factors. For example, such climate trends may result in a disconnect between smolt outmigration timing and food availability.

Specific statistical methods include regression models (to understand which factors best predict the SARs), time series methods (to test for the presence of variations and patterns over time), and correlations (to examine relationships between SAR trends and the predictor variables). Specifically, we will evaluate the usefulness of multiple methods including dynamic factor analysis, principle component analysis, and state-space models. Mixed effects models will also be incorporated where needed, where the random effect (with multiple samples for a given sampling object) is watershed, subregion, or year.

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

3.4 Implications and next steps

This study is designed to answer questions about some of the drivers of spatial and temporal patterns of marine survival of steelhead populations. It will also provide the foundation for additional correlative analyses, discussed in Study 4.

3.5 Timeline

We have already collected SAR and smolt abundance data for a number of wild and hatchery steelhead populations and stocks for ocean entry years 1975-present. We have also begun to examine correlations of the SAR data with changes in smolt abundance. Collection of existing individual fish and population data is needed along with their analyses. We anticipate completing this entire study by early 2015.

3.6 Literature cited

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4) Study 4: Comparing SAR and telemetry data on marine mortality with environmental and buffer prey data

Principal Investigator: Neala Kendall (WDFW)

4.1 Justification

By understanding relationships between SAR values and individual fish and population characteristics, we can better understand the marine and freshwater drivers of these trends. Specifically, if freshwater processes are more closely related to SAR trends than marine ones, this suggests that lower marine survival trends may be driven by fish being weaker as they enter the marine environment. On the other hand, stronger correlations of SARs with conditions and processes in the marine environment shift the evidence towards that stage limiting steelhead abundance and productivity.

4.2 Background and hypotheses

For this study we will relate Puget Sound steelhead SAR trends and mortality data obtained from telemetry studies to environmental and buffer prey data. Environmental data would include Puget Sound temperature, turbidity, pathogens, contaminants, and harmful algal blooms (HABs). Buffer prey data will include abundance, size and age composition data for herring (and other buffer prey alternatives if data are available).

Study 4 addresses the questions:

1. **Which ecosystem indicators best predict steelhead early marine survival?**

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

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

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

4.3 Materials and methods

Existing environmental data including surface temperature, water turbidity, contaminant levels, HAB presence (such as *Chaetoceros* and *Heterosigma akashiwo*, a microflagellate), and potential buffer prey abundance trends will be collected and compiled, and new data such as pathogen concentrations (e.g., *Nanopheytus*) will be collected via other studies in this work plan. Puget Sound SAR and mortality data from telemetry studies will be statistically analyzed and correlated with these environmental and potential buffer prey data, and we will determine if statistical models can be created that predict mortality in various regions of Puget Sound based on the environmental data. This evaluation will be performed at the watershed, sub-region (south Puget Sound, central Puget Sound, north Puget Sound, Hood Canal, Strait of Juan de Fuca), and Puget Sound region level.

As in Study 3, specific statistical methods include regression models (to understand which factors best predict the mortality and SAR values), time series methods (to test for the presence of variations and patterns over time), and correlations (to examine relationships between SAR or mortality trends and the predictor variables). We will again evaluate the usefulness of multiple methods including dynamic factor analysis, principle component analysis, and state-space models. Mixed effects models will also be incorporated where needed, where the random effect (with multiple samples for a given sampling object) is watershed, subregion, or year.

4.4 Implications and next steps

The analyses completed in this study will account for variability in the estimates SAR and mortality estimate and will help determine whether certain characteristics are contributing uniquely to mortality in Puget Sound.

4.5 Timeline

We have already collected SAR data for a number of wild and hatchery steelhead populations and stocks for ocean entry years 1975-present. Telemetry data from 2006-2010 have also been collated. A significant amount of environmental data already exists, collected by the Washington Department of Ecology, University of Washington, WDFW, and others. These data will be combined with new information, and we will perform the correlations and modeling of the SAR and telemetry data with environmental data. The work will be completed by spring 2015.

5) Study 5: Identifying juvenile steelhead predators in the marine environment

Principal Investigators: Scott Pearson (WDFW) for marine birds and mammals and Barry Berejikian (NOAA) for fish

5.1 Overview

WDFW will complete the literature review to identify most likely predators on steelhead smolts. Telemetry data suggest high steelhead mortality occurs in outmigrating smolts while they are in Puget Sound. When examining potential mechanisms driving this apparent mortality, it is important to consider top–down factors such as predation. To evaluate whether or not predators are responsible and the degree of impact, it is important to assess whether or not the pattern of mortality is consistent with predation.

5.2 Objectives/Hypotheses

The following questions will be investigated:

- Who are the potential marine mammal and bird predators?
- Is there an apparent relationship between marine mammal and bird population trends/distribution and steelhead population trends? In other words, are potential predators populations expanding in range and size as the steelhead population has declined?
- Is there a spatial overlap between the identified potential marine mammals and birds and identified hot spots of steelhead mortality in Puget Sound?
- Given this review of predator diet, distribution and population trends, what are the next steps (research and information needs) for identifying and evaluating predation as a potential mechanism for low early marine steelhead survival?

5.3 Approach/Methods

Identifyiing potential marine mammal, avian, and piscine predators

We will review the literature, databases and data in-hand to answer the following questions:

1. Does the predator eat juvenile salmon?
2. Does the predator eat juvenile steelhead?
3. Does the predator feed at the depth steelhead typically outmigrate?
4. Is there an overlap in size distribution between predator diet and that of juvenile steelhead?
5. Is the predator reasonably abundant in Puget Sound and Strait (especially in steelhead mortality hotspots) in May and June when steelhead typically outmigrate?
6. Is the predator population increasing and/or has there been a change in distribution during the time of steelhead decline?

The results of this review will be summarized in a detailed matrix in Excel and the potential steelhead predators will be described in text. By addressing these questions for each predator, we will be able to identify a potential suite of predators whose diet, distribution and behavior is consistent with a predator consuming juvenile steelhead in Puget Sound and the Strait during outmigration.

For the identified potential predators, is there a negative relationship between the steelhead's population and the predator's population or has there been an increase in a predator's use of the areas where high steelhead mortality is occurring?

This helps us address whether or not potential predator populations are increasing and/or has there been a change in distribution during the time of steelhead decline? For this work, we will only include the identified potential predators above. We will compile existing data sets and, when available, examine this relationship quantitatively or qualitatively as appropriate.

Identifying future research needs

Once potential juvenile steelhead predators have been identified, we can also identify future research needs that will help us better understand if predators are responsible for the low steelhead survival at the population level. If predation is an important mechanism for low survival, it can also help us develop management solutions that are likely to enhance survival.

5.4 Product

The results of the literature and data review and any correlations between steelhead and predator abundance and distribution will be summarized in a technical report that will 1) identify potential predators, 2) assess correlations between predators and steelhead, and 3) identify a path for assessing the potential impact of predation on early marine steelhead survival. At the end of this work and if the data compiled are suitable, we will move this work to a peer-reviewed manuscript but additional funds will likely be needed to complete this a peer-reviewed manuscript.

5.5 Timeline

This study is ongoing. The analysis and reporting will be completed by October 2014.

6) Study 6: Genome-wide association study of acoustically tagged steelhead smolts in the Salish Sea: measuring differences between survivors and non-survivors

Principal Investigator: Ken Warheit (WDFW)

6.1 Overview

Genome-wide association studies (GWAS) use genome scans (e.g., a panel of 1000s to 10,000s single nucleotide polymorphism, SNPs) to document a relationship (i.e., association) between a phenotype (e.g., morphological feature, behavior, illness, measure of fitness or survival) and a genotype (e.g., blocks of linked SNPs), based on population samples (McCarthy et al. 2008). Most of the initial GWA studies were designed to discover associations between human diseases and blocks of genes, or to document better quantitative trait loci (QTLs) (McCarthy et al. 2008, Hindorff et al. 2009). Now, with the arrival of relatively inexpensive next-generation sequencing technologies, GWAS and QTLs are used also in a wide variety of studies from understanding diseases in (Purdie et al. 2011) and improving the agricultural production of (Daetwyler et al. 2013) domestic animals and plants, to documenting the genetic basis of horn morphology in wild sheep (Johnston et al. 2011). In salmonids, GWAS have been used, for example, with development rates (Miller et al. 2012) and migratory behavior (Hecht et al. 2012a) in steelhead/rainbow trout, and disease resistance in Atlantic salmon (Houston et al. 2012). In this study, we propose to investigate if there is an association between genomic signatures and survival in outmigrating steelhead smolts in the Salish Sea.

6.2 Methods

Genetics samples have been taken from outmigrating steelhead smolts that have been fitted with acoustic tags, from spawning populations in Hood Canal (Moore et al. 2010) and Nisqually River (Sayre Hodgson, pers. com. 2013) (Table 1). We are interested in comparing the genomic signatures of smolts that are successful in their migration through the Salish Sea and are detected at the final acoustic receiver array at the western end of the Strait of Juan de Fuca, with those individuals that fail to reach the final acoustic array. Since failure to detect an acoustic signal at any one specific receiver array can be the result of either mortality or missed acoustic signal, to reduce the chance of confusing a missed signal with mortality, we limit our samples to (1) smolts that are detected at the final acoustic array (survivors), and (2) smolts that are not detected at any acoustic array (non-survivors). This means, to be classified as a non-survivor, the fish goes undetected at a minimum of two receiver arrays (e.g., Hood Canal bridge and Strait of Juan de Fuca arrays).

We will genotype the survivors and non-survivors using restriction-site associated DNA (RAD) sequencing (RAD-seq). RAD-seq is a genome complexity reduction technique that results in short DNA sequences associated with specific restriction enzymes (Baird et al. 2008). This method generally produces 1000s to 10,000s single nucleotide polymorphisms (SNPs) that can be used for a variety of studies, including GWAS. To eliminate polymorphism associated with the salmonid duplicated genome, and to map SNPs onto established linkage groups, we will align our RAD sequences to those from Hecht et al. (2012), Miller et al. (2012), and Young et al. (unpublished), using only those polymorphisms with 100% alignment. We will use a variety of models to test statistically for a relationship between mapped SNPs and smolt survival. These analyses may include general linear models using as co-factors area, population, year, and any morphological feature measured on these smolts (e.g., length, weight) (see Hecht et al. 2013 for salmonid example). We will also explore the use of formal survival analyses, now used in human health-based GWAS (e.g., Lin et al. 2011).

Table 1. Samples to be used in the GWAS.

Area	Population	Year	Number of DNA Samples	
			No detection	Detected at JDF Line
Hood Canal	Big Beef Creek	2006	10	19
Hood Canal	Big Beef Creek	2007	7	6
Hood Canal	Big Beef Creek	2008	6	2
Hood Canal	Big Beef Creek	2009	14	2
Hood Canal	Big Beef Creek	2010	14	2
Hood Canal	Skokomish R.	2006	12	6
Hood Canal	Skokomish R.	2007	24	5
Hood Canal	Skokomish R.	2008	7	4
Hood Canal	Skokomish R.	2009	15	3
Hood Canal	Skokomish R.	2010	7	0
South Puget Sound	Nisqually R.	2006	13	8
South Puget Sound	Nisqually R.	2007	16	3
South Puget Sound	Nisqually R.	2008	2	0
South Puget Sound	Nisqually R.	2009	37	2

6.3 Timeline

Feb – March 2014	Obtain all samples and extract DNA
April – Jun 2014	Build RAD libraries
July 2014	Progress report #1
Jul 2014 – Sep 2014	RAD sequencing
Sep – Dec 2014	Bioinformatics and data analysis
Dec 2014	Progress report #2
Jan – May 2015	Final data analysis and report preparation
May 2015	Final report

6.4 Literature Cited

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7) Study 7: Conduct a Puget Sound-wide fish health assessment of outmigrating steelhead

Principal Investigators: Paul Hershberger (USGS) for pathogens and Sandie O'Neill (WDFW) for toxic contaminants

7.1 Background and hypotheses

The purpose of this project is to conduct a field assessment of juvenile steelhead health as they migrate from fresh water habitats through the marine systems of Puget Sound. Per section 3.3.1 in the body of this report and the complete write up in Appendix 2, a group of regional fish health specialists concluded that *Nanophyetus salmincola* is the pathogen that most likely accounts for the observed mortality patterns in Puget Sound steelhead. Other pathogens were also discussed and ranked with either moderate or low probability of contributing to juvenile steelhead mortality in Puget Sound. The infection prevalence and intensity of *Nanophyetus* or other potential pathogens of steelhead could be enhanced if steelhead are exposed to contaminants in freshwater, estuarine and marine habitats of Puget Sound, as discussed in section 3.3.2 in the body of this report.

We propose to conduct a synoptic scan of pathogens and toxic-contaminants in outmigrating steelhead at their major rivers, estuaries and the marine basin of Puget Sound to assess whether steelhead smolts are exposed to sufficient levels and types of pathogens (especially *Nanophyetus*)or toxic chemicals as they migrate into Puget Sound that could reduce their marine survival.

Exposure to pathogens and toxic contaminants could reduce marine survival directly, or indirectly, by reducing their health and increasing susceptible to predation. The study tests the following hypotheses:

<u>Hypothesis 1:</u> The infection prevalence and intensity of pathogens (especially *Nanophyetus*) is higher in juvenile steelhead populations sampled from more southerly watersheds of Puget Sound compared to northerly populations.

<u>Hypothesis 2:</u> Outmigrant juvenile steelhead in developed rivers of the Central Puget Sound and those migrating through urbanized waters of the Central Puget Sound basing are exposed to higher toxic contaminant levels than fish in the North Puget Sound or Hood Canal.

The null hypotheses are that there is no difference between pathogen and toxic contaminant exposure in steelhead across in-river sampling locations and between fresh- and saltwater habitats within a river-estuary-basin sampling complex.

7.2 Expected outcome

Following the infection prevalence and intensity of *Nanophyetus* in cohorts of outmigrating steelhead will indicate whether infected individuals could experience mortality during outmigration and early seawater entry. Assuming juvenile steelhead can be captured in the marine environment, some indication of whether the disease leads to mortality in infected migrants will be provided by examining the progression of infection prevalence and intensity (by histology) as the smolts migrate. If the results support the hypothesis that infected individuals experience elevated mortality, then additional controlled studies (e.g., live box studies, laboratory pathogen exposures) will be recommended to address cause-and-effect. Assessing the contaminant exposure in wild juvenile salmon in the same cohorts of fish can be used to assess whether pathogen infection prevalence and intensity is exacerbated by exposure to contaminants. Tissue residues will also be compared with published effects thresholds to evaluate the potential health effects on juvenile steelhead from exposure to contaminants. Finally, gut contents and tissues will be preserved for evaluating diet composition and the presence of other pathogens, respectively, if it is deemed valuable in the future.

7.3 Study design

7.3.1 Fish Sampling Location and Collection Method

We will sample juvenile steelhead from up to five major watersheds within Puget Sound: 1) the Skagit River and estuary, 2) the Snohomish River and estuary, 3) the Green/Duwamish River and Elliott Bay, and 4) the Nisqually River and estuary, and 5) the Tahuya River and estuary (Table1). These river systems represent steelhead entering Puget Sound from various sub-basins, and their watersheds encompass a range of land-use practices from relatively undisturbed areas such as the Nisqually and Tahuya, to agricultural regions such as the Skagit, to heavily urbanized areas such as the Green/Duwamish/Elliott Bay. The Green, Skagit and

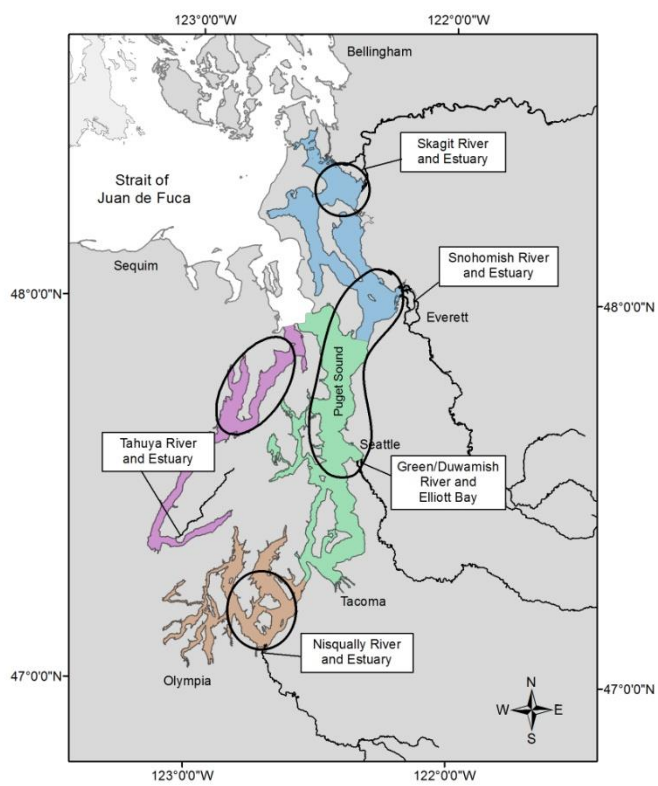

Figure 1. Sampling locations for the steelhead health assessment.

Nisqually watersheds and Southern, Main, and Whibey marine basins are priority locations identified for alignment with the reciprocal transplant study and to evaluate the south to north variation in early marine mortality in Puget Sound.

Within each river/embayment, salmon will be sampled at hatcheries, at smolt traps in the lower river,at existing fyke net/ beach seine and surface trawl locations in the estuary and in adjacent marine water using purse seines, if feasible. We will consider evaluating steelhead in net pens or cages in the marine environment, for location-specific effects, if marine capture is not feasible.

Hatchery and wild steelhead will be collected at each sampling site as detailed in Table 2, below; however the toxic contaminant scan will target wild fish and will focus on the priority locations referenced in the previous paragraph.

7.3.2 Pathogen Scan

The primary focus of the pathogen scan will be an assessment of the infection prevalence and intensity of *Nanophyetus* in juvenile steelhead. At each sampling site, 30 hatchery and 30 wild fish, as detailed in Table 2 will be examined for the presence of *Nanophyetus.* The primary screening test method is enumerating the number of cysts in the posterior 1/3 of kidney using light microscopy. Tissue of kidney, heart, liver, and gill will also be collected and fixed in Davidson's solution from all sampled fish for subsequent histology examination. Tissue for these samples will be archived and available for future testing to assess pathological damage and host immunological response associated with *Nanophyetus* infections. Additionally, samples of liver, kidney and gonad will be samples and stored in RNAlater (or EtOH) for potential future analyses with by PCR to assess the presence of other lower priority pathogens that are not well sampled by the above methods (e.g. bacterial pathogens – see Table 1 for details).

No evidence exists to indicate that steelhead smolts are capable of fully clearing or recovering from *Nanophyetus* infections; therefore, any decrease in Nanophyetus prevalence as the smolts out-migrate will be assumed to result from either direct or indirect (predation targeted on infected cohorts) mortality from the parasite. Differences in parasite prevalence throughout a single watershed will compared using the Chi Square statistic.

7.3.3 Toxic Contaminant Scan

Steelhead collected for the pathogen scan (discussed above) will be also be used to assess the extent to which juvenile steelhead are exposed to toxic contamiants. Prior to removing fish's internal organ samples for pathogen analyses, biles samples will be extracted from individual fish to analyse for the presence of PAH metabolites, xenoestrogens, and selected pharmaceutical (i.e. anti-depressents). Subsequently, bile from individual fish will be and combined to create composite samples of 5 fish each. Whole body samples of fish (less bile, internal organs sampled for pathogens, and gut content) will be retained and analyzed for the presence of peristent organic pollutants (PCBs, PBDEs, and chlorinated pesticides) and lipids. Prior to analyses, fish carcasses will be combined in composite samples of 5 fish each. As detailed in Table 2 up to three composite bile and whole body samples will be collected at each sample site but for this proposal, analyses of samples will be limited to wild fish and in smolt traps and estuary of the Skagit, Green and Nisqually systems and the hatchery or wild fish collected in Whidbey, Cental and Sound Sound basins. Samples will be maintained on ice during the necropsy procedure, and then transferred to -20° C or colder freezers for storage at the NWFSC laboratory in Seattle.

7.3.4 Ancillary Data Collection

Fish lengths and weights will be recorded for all sampled fish prior to the necropsy work for the pathogen and toxic contaminant scan. Also fish fin snips will be collected and stored individually in ethanol for subseqent genetic stock identification. During the necropsy process, otoliths and scales will be collected for aging and gut contents will be removed and preserved for subsequent diet analyses, if desired.

7.3.5 Saltwater adaptation of *Nanophyetus*: infected vs uninfected hatchery steelhead smolts

A group of 30 hatchery steelhead smolts from the Green River (Soos Creek Hatchery) and a group of 30 hatchery steelhead smolts from the Skagit River (Marblemount Hatchery) will be transported live to the US Geological Survey, Marrowstone Island Laboratory. Transport will be done on separate days in order to closely match both groups in actual time of transport. Once at Marrowstone Island, both groups will be adapted to seawater over an 8 hour period and monitored for health over a 60 day period by Marrowstone Island staff. Fish that show acute (first 48 hours) or chronic illness will be removed and sampled for histopathological examination. *[This element of the study was added as a backup measure for evaluating the potential effects of nanopheytus as steelhead travel into the marine environment. The backup measure was added due to concerns about the number of steelhead that will feasibly be captured in the estuary and marine environments.]*

Table 1. Sampling locations and numbers of juvenile steelhead to be collected and analyzed for a pathogen and toxic contaminant scans. Pathogen scans will be conducted on individual fish. Contaminant scans will be conducted on a subset of the fish, three composite samples per site, each composed of 5 fish. **Priority sites and samples for contaminant scal are noted in bold, underlined text.** Lower priority sites and samples for contaminant analyses are will be collected as time allows and archived for potential future analyses.

		Number Individual Fish to be Sampled[1]			Fish Pathogen Scan Samples[2]			Contaminant Scan Samples[3]		
Study Location	Collection Area	hatchery	wild	hatchery or wild	hatchery	wild	hatchery or wild	hatchery	wild[#]	hatchery or wild[#]
Nisqually River*	Hatchery									
	Smolt Traps		30			30			**3**	
	Estuary	30	30		30	30		*3*	**3**	
Southern Basin*	Offshore			30			30			**3**
Green River*	Hatchery	30			30			*3*		
	Smolt Traps	30	30		30	30		*3*	**3**	
	Estuary	30	30		30	30		*3*	**3**	
Snohomish River	Hatchery	30			30			*3*		
	Smolt Traps	30	30		30	30		*3*	*3*	
	Estuary	30	30		30	30		*3*	*3*	
Main Basin*	Offshore			30			30			**3**
Skagit River*	Hatchery	30			30			3		
	Smolt Traps	30	30		30	30		3	**3**	
	Estuary	30	30		30	30		3	**3**	
Whidbey Basin*	Offshore			30			30			**3**
Tahuya River	Hatchery									
	Smolt Traps		30			30			*3*	
	Estuary	30	30		30	30		3	*3*	
Hood Canal Basin	Offshore			30			30			*3*

***** Priority sites identified for alignment with the reciprocal transplant study and to evaluate the south to north variation in early marine mortality in Puget Sound.

[1]Lengths and weights of each fish will be measured, and ancillary samples (gut content, fin snips, otoliths and scales) will be collected. See text for details.

[2]Pathogen Scan includes: 1) collection and analyses of kidney tissue for microscopic scan of *Nanophyetus salmincola* 2) collection and storage of kidney, gill, heart, and liver tissue in Davidson's solution for potential future histological analyses for medium and low priority pathogens, 3) as time allows, collection and storage of kidney, gill, and liver tissues in RNAlater or EtOH for potential future PCR analyses for medium and low priority pathogens, and 4) visual scan of the gill tissue for the presence of sea lice. Histological analyses will be completed for any fish that test positive for *N. salmincola* or other symptoms of disease. See text for details.

[3] Contaminant Scan includes collection of 1) whole bodies (less gut contents and partial sampling of gill, kidney and liver samples) for persistent organic pollutants and lipids, and 2) bile samples for the presence of PAHs metabolites, synthetic and naturally occurring estrogens, bis-phenol A, nonyl-phenol, and selected pharmaceuticals. See text for details. Up to three composite bile and whole body samples, composed of five fish each, will be collected at each sample site but **for this proposal, analyses of samples will be limited to priority sites (Nisqually, Green, and Skagit systems) and priority samples (wild fish in smolt traps and estuary of the Skagit, Green and Nisqually systems and the hatchery or wild fish collected in Whidbey, Cental and Sound Sound basins).**

[#] wild samples in smolt traps and estuaries and hatchery or wild samples in offshore sites are highest priority sites for contaminant scan analyses. However, if sufficient wild fish are not not collected, analyses will conducted on hatchery fish.

7.4 Timeline

All samples will be collected in the 2014 spring/summer outmigration period for winter steelhead. We anticipate sampling to commence in late April and continue into June. Samples will be timed to match the peak outmigration run, as best judged by the area biologists working in these systems. Analyses will occur through the fall of 2014, and reporting will be completed by June 2015.

7.5 Potential Constraints

This study would require lethal sampling of ESA-listed steelhead and possible handling of other ESA listed salmonids which may constrain the number of fish we are allowed to take. The full sampling design for 5 river-estuary systems and 4 marine basins of Puget Sound could require a lethal take of up to 750 fish, including up to 300 wild fish. To reduce by-catch of ESA listed Chinook in estuarine and marine waters, nets used to capture steelhead will be a larger mesh size to preclude the capture of the smaller Chinook salmon.

Another possible constraint is the availability of fish and amount of bile produced by individual fish. Although migration timing is well known for this species, abundance varies spatially across a relatively short window of opportunity. Sampling is constrained to this window. Successful analysis for PAH metabolites, xenestrogens, and selected pharmaceuticals will be constrained by collection of a sufficient volume of bile. We may need to sample up to 10 individuals per composite sample to obtain sufficient volume for the PAH analyses.

7.6 Deliverables

In addition to a final project report, detailing the results from the fish health study, we expect the results from the *Nanophyetus* surveillance to be submitted for publication in a peer-reviewed scientific journal in 2015. Likely outlet: 'Prevalence and intensity of *Nanophyetus salmonicola* in Puget Sound steelhead. Diseases of Aquatic Organisms.'

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8) Study 8: Perform a reciprocal transplant study to partition population effects from Puget Sound location effects

Principal Investigators: Barry Berejikian and Megan Moore (NOAA)

8.1 Justification

The steelhead marine survival project is proceeding under the premises that predation is the primary proximate cause of mortality for steelhead smolts as they migrate to the Pacific Ocean and that mortality during this period is a primary factor limiting their overall marine survival, productivity and abundance. Minimum survival estimates for smolts from their respective river mouths to the western Juan de Fuca Strait hover around 15-20%. Even if survival rates are twice the telemetry-based estimates, approximately 60 to 70% of the smolts are dying with an approximate two-week migration period (Moore et al. in prep) through Puget Sound. While high mortality in anadromous salmonids soon after marine entry is expected, the estimated survival rates are consistent with low smolt-to-adult survival rates estimated for Puget Sound steelhead populations and particularly steep declines in abundance in central to southern Puget Sound populations (Green, Puyallup, Nisqually populations) since the mid 1980s.

8.2 Objectives

An on-going analysis of acoustic telemetry data collected from 2006 through 2009 indicates that Nisqually River smolts experienced higher 'initial' marine survival from the estuary to the Tacoma Narrows Bridge than Green River smolts did from the Duwamish estuary to the next telemetry line in Central Puget Sound (Figure 1). Nisqually smolts subsequently had lower survival from Tacoma Narrows to Central Puget Sound than in any other segment earlier or later in their migration. These patterns suggest a mortality ‘hotspot’ in Central Puget Sound. Because the populations may differ in their ability to survive migration through Puget Sound (population effects include genetic differences or rearing environment effects prior to the time of migration), a reciprocal transplant study will be performed to test two primary hypotheses to partition population effects from Puget

Sound location effects. The hypotheses follow from analysis of telemetry data from 2006 through 2009 (Moore et al., in prep).

H_{01}: The Green River population suffers greater marine mortality than the Nisqually River population, independent of where they enter Puget Sound.

H_{02} : Mortality through Central Puget Sound is greater than through South Puget Sound, regardless of population of origin (Green and Nisqually).

An additional hypothesis will be tested in conjunction with the above hypotheses.

H_{03} : Individual fish condition (fork length, weight, condition factor) and migration timing have no effect on early marine survival.

8.3 Materials and Methods

8.3.1 Data Collection

Receiver network

Vemco VR3 acoustic telemetry receivers will be deployed at appropriate spacing to detect the great majority of steelhead smolts migrating from their natal rivers, through Puget Sound to the Pacific Ocean. Lines or groups of receivers will be deployed: i) in the Green/Duwamish and Nisqually River estuaries, ii) across Puget Sound approximately 25 km north of the Nisqually River in the Tacoma Narrows (TAC), 25 km north of the Green River in Central Puget Sound (CPS) iii) across Admiralty Inlet (ADM), and iv) across the Strait of Juan de Fuca (JDF) at Pillar Point (already deployed, Figure 2). Spacing between adjacent recievers in the TAC and CPS lines will be similar to spacing in the Admiralty Inlet line (300 - 400 m). Receivers will be deployed in February or early March. Telemetry-tagged smolts will be detected at each of these locations and will be individually identified (see below).

Smolt tagging and transport

Steelhead smolts will be captured in screw traps operated by the WDFW in the Nisqually River and Green River. Half of the smolts collected in each river will be implanted with acoustic telemetry transmitters and released into their river of origin and the other half will be transferred to the other river, implanted, and released (i.e., a reciprocal transplant). Based on historic outmigration timing, tagging should begin in early May and continue through the end of May; however tagging may begin as early as April 15 and end as late as June 18. A total of 240 fish will be tagged, including 120 from each population. Every sixth fish will receive a transmitter that is programmed to be off for the first 10 days test the 'dinner bell' hypothesis (a.k.a. Study 10).

Each week the goal will be to collect 30 smolts at each trap. Captured smolts will be placed in 1.2-m diameter covered holding tanks supplied with river water and aeration from an AC-powered air bubbler. Smolts collected on day 1 will be placed into a single vessel. If 30 smolts are captured, they will be tagged (Vemco V7 2L acoustic transmitter, 1.7 g according to the protocols described in Moore et al. 2010) on the day of capture and either returned to their vessel and held overnight or transported to the reciprocal location and held overnight. If a second day of collections is necessary to obtain 30 smolts, those collected on the second day will be placed into a separate vessel, and all smolts (vessel containing day-1 smolts and vessel containing day-2 smolts) will be tagged on the same day and either returned to their vessel or transported to the reciprocal location. To minimize variation caused by differences among surgeons, the same two surgeons will conduct all surgeries and tag implantation. The two surgeons will tag equal numbers of fish from each of the four groups (Green-home release, Green-Nisqually release, Nisqually-home release, Nisqually-Green release).

Smolts will be transported in a 400 L insulated vessel supplied with battery powered air bubblers. Transport densities will be approximately 5 g of fish biomass per liter of water. All smolts will be held in vessels at their river of release overnight to allow for acclimation and recovery from tagging. Releasing smolts into the Green River will require a transport of approximately 30 min from the Green River trap site to the Green River release site. Thus, smolts to be released into the Nisqually River will be transported for the same duration (30 min) to equalize transport times. Releases should occur in the evenings at approximately the same time.

8.3.2 Data recovery

Data will be downloaded from the various receiver arrays in August 2014 after all smolts have migrated. A VR3 modem will be used to 'awaken' each receiver and download the detection data. It is estimated that the Admiralty Inlet, CPS and TAC data can be downloaded in three to four days. VR2W receivers deployed in the Duwamish River estuary will be removed from moorings, and Nisqually Delta receivers will be removed by grappling. Data from VR2W receivers will be downloaded directly to a PC.

8.3.3 Data analysis

Receiver detection data for each individual smolt will be incorporated into mark-recapture models to estimate survival and detection probability at each receiver line and to test factors affecting survival (see Figure 2). The main effects to be included in the mark-recapture models will include: population, early marine segment, and individual metrics (condition, timing). Cormack-Jolly-Seber (CJS) mark-recapture methodology will be used to estimate apparent survival probabilities for each segment, and detection probabilities at the river mouths (RM), TAC, CPS, ADM and JDF (Figure 2). The R (R Development Core Team 2007) package RMark will be used to construct survival (s) and detection probability models for the program MARK. Models will incorporate data from all 240 tagged individuals. Goodness-of-fit of the detection data to the CJS models will be tested using the program RELEASE (within MARK). One important issue with the CJS model is the inability to distinguish between mortality and emigration, so in this study, 1- s represents both animals that died and those that did not migrate. This issue generally tends to cause underestimation of survival. Unique combinations of grouping and continuous variables will be used to construct a series of models to be tested in RMark. Akaike's Information Criteria (AIC) will be used to identify the set of variables that parsimoniously explains the variation in the survival and detection data. Modeling results will be adjusted using the estimated variance inflation factor (c) to compute QAICc values, which are adjusted AIC values that compensate for extrabinomial variation and small sample sizes.

To test the effects of transmitter sound on survival of steelhead smolts, a subset of tags (N = 40, 10 per treatment) would programmed to be initially silent then turn on prior to the time smolts would reach the furthest telemetry line at Pillar Point. Survival of fish to the Strait of Juan de Fuca line will be compared between fish with tags that were always pinging to fish with tags that were silent for approximately 7 to 10 days (to be determined)

8.4 Implications and next steps

This study is designed to answer questions about where, when, and how quickly steelhead smolts die as the migrate from their natal rivers, through river mouths and through Puget Sound. It will also inform whether survival of the two populations differs during their downstream migrations and migrations through Puget Sound. It is not designed to inform how they are dying, but may confirm that predation is a primary cause if mortality occurs quickly as indicated by previous studies. This study does provide the tagged steelhead smolts necessary to evaluate steelhead-seal interactions; so in that way, it also addresses one potential cause of predation (predation by harbor seals).

If early marine segment (first 25 km) significantly affects survival (e.g., if RM to CPS survival is significantly lower than RM to TAC survival, then the next step would be to focus on factors that are different in CPS than SPS (water quality, predator assemblages, algal blooms, etc). If a common hotspot is detected for both populations (e.g., CPS to ADM), then the next step may be to identify conditions in that segment that differ from Central and South Puget Sound. If significant population effects are detected, then next steps may include closer investigation into the freshwater rearing environments experienced by smolts for the 1-3 years prior to their migration and potential genetic mechanisms that may influence marine survival, which may also be informed by the GWAS study. Smolt trapping data suggest differences in outmigrant timing between the two populations, so effects of outmigration timing on marine survival may unveil important mechanisms and help explain any between-population differences in survival.

In short, this study will improve understanding, of who, where, when and how quickly steelhead smolts die in Puget Sound, and will contribute to narrowing the list of potential mortality agents. Follow up investigations may include experimentally testing the effects of freshwater quality, Puget Sound water quality, or more closely investigating predator prey interactions in more precise locations and times.

8.5 Timeline

Activity	Start date
Purchase batteries	August 2013
Permitting	August 2013
Purchase tags and tagging supplies	January 2014
Surgery training and preliminary tag effects assay	February 2014
Deploy VR2W receivers in estuaries	March 2014

Deploy VR3/4 receivers	March 2014
Download VR3 and recover VR2	August 2014
Data Analysis	Sept 2014
Reporting	January 2015

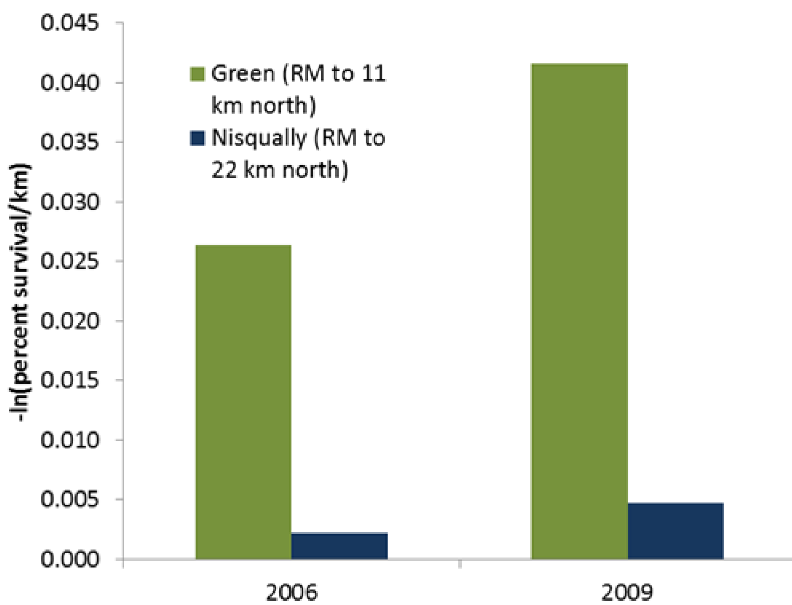

Figure 1. Instantaneous mortality rate (by distance) for Green River smolts migrating from the river mouth to the telemetry line located 11 km north, and Nisqually River smolts migrating from the river mouth to 22 km north. Differences may have reflected population effects, differences in marine conditions between the two segments or a combination of the two factors. The present study is designed to separately estimate the effects of population and marine segment.

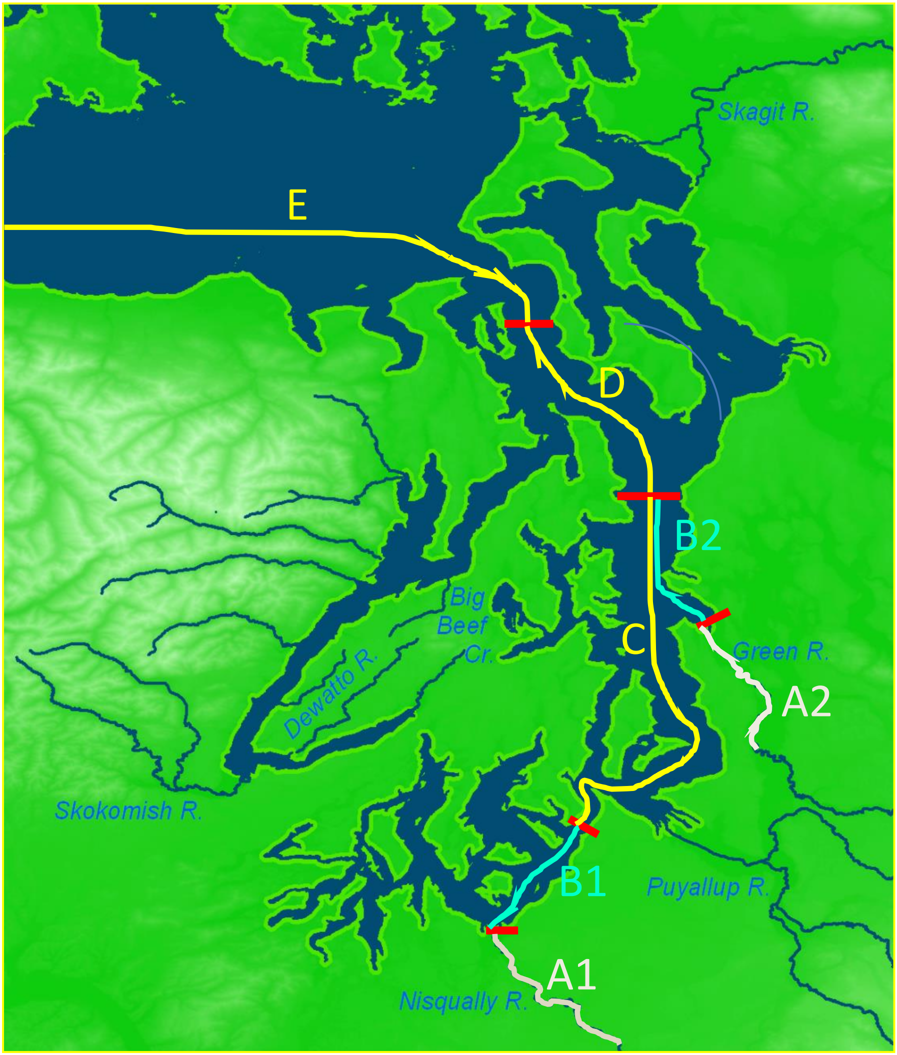

Figure 2. Segments for which survival will be estimated. For smolts released in the Green River, survival will be evaluated in freshwater (A2), and in three separate marine segments (B2, D, and E). Survival for smolts released in the Nisqually River will also be evaluated in freshwater (A1), but will be estimated for four marine segments (B1, C, D, and E). To facilitate comparison between congruous segments (e.g., A1 and A2 or B1 and B2), two separate models will be constructed. One model will investigate effects on survival in A and B, and a second model will test factors influencing survival through D and E. Akaike Information Criteria (AIC) will be used to identify the single factors and/or interactions within our candidate model set that best explain the variation in survival among release groups.

9) Study 9: Evaluating interactions between harbor seals and steelhead smolts in Puget Sound

Principal Investigators: Steve Jeffries (WDFW), Barry Berejikian and Megan Moore (NOAA)

9.1 Justification

Increases in harbor seal populations in Puget Sound and concomitant declines in a number of harbor seal prey species (e.g., Puget Sound herring and hake) have occurred during a period of reduced marine survival and abundance of Puget Sound steelhead. Harbor seals are known to prey on steelhead smolts in some areas, but predation events are difficult to observe. Harbor seals are the only marine mammal in Puget Sound considered to be at relatively high abundance in Puget Sound year-round (Gaydos and Pearson 2011). Even if steelhead constitute a small percentage of the harbor seal diet that is difficult to detect by direct observation or scat analysis, predation by harbor seals may still constitute a significant source of steelhead mortality. Movement patterns from acoustic telemetry tags implanted into steelhead smolts are suggestive of tags inside predator stomachs in localized regions of the Salish Sea (Melnychuck et al. 2013, Moore et al. 20013). The proposed study takes advantage of the reciprocal transplant experiment (study #9) that will implant approximately 260 acoustic telemetry transmitters in steelhead smolts in the Nisqually and Green Rivers and the fixed telemetry receivers in Puget Sound to provide timing and location information for each detected smolt.

9.2 Objectives

This study attempts to quantify spatial and temporal overlap of harbor seals and steelhead smolts in Puget Sound and potentially document predation events through the use of acoustic telemetry and other tagging methods.

The specific objectives of the study are to:

- Quantify core foraging areas of harbor seals during the steelhead smolt outmigration;
- Quantify the spatial and temporal overlap of harbor seals and steelhead smolts in specific areas of Puget Sound; and
- Quantify predation events by seals on tagged steelhead smolts

9.3 Materials and Methods

9.3.1 Steelhead tagging and detection on fixed arrays

Steelhead tagging and tracking-- Steelhead will be tagged during the smolt outmigration period (May) in the Nisqually and Green R (see study 9). Each smolt will be implanted with Vemco V7-

2L transmitters designed to emit an acoustic ping at a nominal interval of 60 s (random intervals between 30 – 90 seconds). A line of receivers will be located at the southern end of the Tacoma Narrows (TAC), across Central Puget Sound from Pt Jefferson to Spring Beach (CPS) and in the northern end of Admiralty Inlet (ADM). Telemetry transmitters carry a unique code that will be detected by the stationary receivers as the smolts swim past. Survival estimation, migratory pathways, travel times and other behavioral measures will be estimated as described in Study 9)

Seal tagging – Harbor seals will be captured by seine and instrumented with a tag package consisting of a GPS transmitter (Wildlife Computers MK10 TDR with Fastloc GPS), a Vemco VMT transceiver, and an ATS VHF tag. The instrument package will be glued between the shoulder blades directly onto the seal's fur. Twelve harbor seals would be instrumented in Central Puget Sound and Admiralty Inlet, which are areas previously associated with high steelhead mortality rates. Kilisut Harbor and Blakely Rocks are two known seal haul-out locations where substantial numbers of seals should be available for tagging (if 12 seals can be captured at Blakely Rocks, then all 12 seals will be tagged there, because steelhead smolts migrating through this area of Puget Sound appear to suffer greater mortality than in other areas). Tagging seals in these locations provides the opportunity to detect interactions between smolts released from both locations (Nisqually and Green) and the seals. Each location is near a receiver line proposed for the reciprocal transplant experiment. The GPS tag will provide detailed positions of harbor seals throughout the study. The VMT transceiver will detect steelhead smolts with pinging tags within a range of approximately 200 m, and it will transmit a signal that can be detected by other transceivers on other seals and on fixed telemetry receivers. Thus, the locations of seals will be known from GPS coordinates and from detections on the receiver array that is detecting locations of steelhead smolts.

Detection range of the VMT receivers – One or two individual V7-2L transmitters will be deployed in Puget Sound near the seal haul-out areas where the seals were captured and tagged. Distances from GPS locations at the time the stationary V7 transmitter is detected by a VMT receiver to the GPS location of the stationary tag will allow us estimate the maximum range and variability in range that the V7 transmitters can be detected by the VMT receivers. The inferred track of each seal can be interpolated between successive GPS locations to provide a fairly accurate estimate of the distance between the seal and the fixed tag at the time each fixed tag is detected by a VMT. Detection ranges of the VMT will be used to infer the maximum distances steelhead smolts may have been swimming from a VMT-carrying seal when each detection of a smolt on a VMT occurred.

9.3.2 Data recovery

Data from the TAC, CPS, ADM and JDF (operated by the Ocean Tracking Network) receivers will be uploaded remotely by a Vemco VR3 modem after the transmitter batteries have expired (approximately 90 days post-tagging). Seal tag packages will be released when the seals molt in the autumn. The GPS locations will be used to identify areas for released tag packages and VHF transmitters be used to pinpoint the locations of each package. Data will be downloaded from the recovered VMT receivers.

9.3.3 Data analyses

Harbor seal foraging areas will be calculated from GPS locations. Locations within 500 m of haul-out locations will be excluded from the foraging area calculation (Thomas et al. 2011). Fixed kernel density estimates will be used to calculate utilization distributions for each harbor seal following approaches described in Thomas et al. (2011). Depending the actual dates of tagging we may be able to compare utilization distributions before (March to Mid April) vs during (Mid April through June) the smolt outmigration period to explore whether any changes in foraging areas are associated with increases in the number of smolts entering Puget Sound. Data from telemetry-tagged smolts passing the receiver arrays and smolt traps in the Green River and Nisqually River will be used to estimate temporal changes in smolt abundance.

Predation events will be inferred from continuous repeated detections of a steelhead smolt on a VMT. We expect that detection frequencies on VMT receivers will be strongly bi-modal, with most unique tag codes being detected infrequently (only a few times), and a smaller number of detections will be repeated continuously for hours or days (depending on gut retention of ingested tags). Such bimodality in detection data was useful in inferring mortality of steelhead smolts at the Hood Canal Bridge (Moore et al. 2013). Weaker evidence of predation events will include tags continuously detected within range of a fixed receiver indicating a tag on the seafloor together with periodic detections on a seal-mounted VMT over a long period of time, but always occurring in the same location. Predation events can also be inferred by tag movements in excess of speeds achievable by steelhead smolts, which indicate the tags have been ingested and are being carried by a predator (Melnychuck et al. 2012)

Logistic regression analysis will be used to determine whether the odds of detection at the next receiver line depend on spatial-temporal overlap with tagged harbor seals. For example, we will tabulate the number of different VMT receivers that detected it each smolt, and the total number of detections for each smolt on VMT receivers. These two variables will be included in the logistic regression to estimate the odds of detection on a subsequent receiver array.

9.4 Timeline

The asterisks indicate activities that are also required for the reciprocal transplant experiment, which supplies the tagged steelhead needed for this study

Activity	Start date
VR3 deployment permitting*	October 2013
Fixed acoustic receiver preparation	October 2013
Purchase tags, tagging supplies, and assemble packages	January 2014
VR3/4 Receiver deployment*	February 2013
Practice tagging and preliminary tag effects assay*	February 2014
Deploy VR2W receivers in estuaries*	March 2014
Deploy VMT receivers	March 2014
Smolt collections, tag, transfer, release*	May and June 2014
Download VR3 and recover VR2*	July 2014
Recover tag packages	October 2014

Data analysis	November 2014
Reporting	January 2015

9.5 Deliverables

The results of this study will be published in the peer-reviewed scientific literature and presented at regional meetings focused on steelhead conservation and recovery in Puget Sound. Results and conclusions will be reported in a manner that provides clear recommendations for next steps. For example, if harbor seals and steelhead are found to be temporally and spatially segregated during the smolt outmigration period and there is no evidence that tagged steelhead were consumed by VMT-fitted seals, then the recommendation may be to focus future investigation other causes of steelhead marine mortality (e.g., other predators, pathogens, etc). If there is strong spatial and temporal overlap and significant evidence of direct predation by VMT-fitted seals and tagged steelhead, the next step would include number of approaches to extrapolate the potential predation impact of harbor seals on steelhead from the two study populations that include modeling consumption rates, harbor seal energetic requirements, seasonal movement patterns, and prey abundance. The expectation is that an addition year or two of intensive research would then be needed to improve confidence in predation rates by harbor seals on steelhead in Puget Sound.

10) Study 10: Testing the dinner bell effect

Principal Investigators: Barry Berejikian and Megan Moore (NOAA)

No additional description provided. See paragraph overview in the Research Components section of this report and Study 8: reciprocal transplant description, above.

11) Study 11: Ecosystem and bioenergetics modeling

Principal Investigators: Chris Harvey (NOAA) and others who are part of affiliated efforts.

No additional description provided. See paragraph overview in the Research Components section of this report.

Appendix 4: Responses to reviewer comments

The following are excerpts from comments to the August 2013 draft work plan where the Workgroup concluded direct responses were warranted. Comments were largely provided informally; therefore, the complete texts of the comments are not included in this report.[30]

1) Puget Sound Salmon Recovery Implementation Technical Team (RITT)

The RITT has a few suggestions for the final steelhead research work plan (SRWP):

1. The Executive Summary and Introduction make the case that low marine survival is a significant factor affecting abundance and viability of Puget Sound steelhead. Although the RITT does not disagree with this statement, we also wish to caution the Workgroup that freshwater factors are also contributing to the decline of steelhead. We recommend the Workgroup consider revisions that acknowledge a comprehensive analysis of effects of freshwater and marine factors is needed and that this Workplan is comprehensive examination of just one phase in the life cycle of Puget Sound Steelhead.

   Response: *The report's introduction has been edited to address this comment.*

2. We suggest the Workgroup expand their list of potential predator species in Study 5 to include piscine predators. That would ensure a comprehensive examination of potential predators. Specifically, the Workgroup should consider reviewing information regarding WDFW's "Winter Blackmouth" Sport catch recreation program. The Puget Sound Recreational Fishery Enhancement (PSRFE) program has been in existence since the early 90's (1993), which appears to correlate with the downward trend in Puget Sound Steelhead Marine Survival. The PSRFE program has a goal of releasing three million delayed-release yearling Chinook per year from locations in Puget Sound and Hood Canal. Delaying the release of those fish from hatchery facilities makes them less likely to leave the Sound, where they are available to sport fisheries throughout the year. It would be prudent to discuss this issue because it is a potential "unnatural piscivoris condition" that has been introduced into Puget Sound and Hood Canal.

   Response: *Piscine predators have been cursorily reviewed in the process of developing this document; however, this wasn't reflected to a great extent in the report. The original assessment table is provided. Note that, where there is evidence, any potential piscine predator population has been declining in abundance over the same time period as the decline of Puget Sound steelhead smolt-to-adult (indicative of marine survival) rates. And, as noted in the report,*

[30] Contact Michael Schmidt, Long Live the Kings, if you are interested in more details about the comments provided (mschmidt@lltk.org).

steelhead tend to migrate near the surface and away from where most of their potential piscine predators would likely hunt.

Regarding resident Chinook, it's difficult to say how "unnatural" a piscivoris condition was caused by yearling hatchery Chinook releases. As you are likely aware, there is an extensive record indicating that resident Chinook (and coho) were historically commonplace in Puget Sound and the Strait of Georgia (Smith 1920; Pressey 1953; Haw et al. 1967)[31]. Research also indicates that a combination of larger freshwater outmigrant size and distance from the Pacific Ocean drive residency more than just larger outmigrant size (Chamberlin 2011)[32]. Furthermore, yearling hatchery Chinook production increased since the 70's, not simply when the PSRFE came into existence, and then has been drastically cut since the early to mid 2000s in response to dramatic declines in smolt-to-adult survival rates, similar to the declining trajectory as Puget Sound steelhead (see Figure 24, below). Finally, resident Chinook tend to be deep water oriented whereas steelhead outmigrate very near the surface. If anything, it appears that both resident Chinook and steelhead have been similarly affected by some survival driver.

As part of the Salish Sea Marine Survival Project, scientists are attempting to get a better handle on the overall proportion of Chinook that express a resident life history, and how that truly has changed over time. And, resident diets and their impact as predators on juvenile Chinook will be evaluated to an extent as part of a juvenile Chinook and coho survival study led by Dave Beauchamp (U. Washington) and Josh Chamberlain (NOAA). See the U.S. Salish Sea Technical Team's Hypotheses and Preliminary Recommendations (2012) report and the soon to be released Research Plan for more information (www.marinesurvivalproject.org on the publications page).

Taxa	Known to eat ***juvenile*** salmon (SA) or steelhead (ST) or similarly sized other fish prey (OP)	Predator population trends (up, down, no change) since mid 1980's	Notes
FISH			Steelhead smolts 140 – 220 mm, so minimum body size threshold for piscine predators would be somewhere around 300 – 400 mm, assuming predators can consume prey up to 50% of their body length.
Bull	SA, OP	Decrease	Capable of consuming steelhead and present in Puget Sound, but largely nearshore areas. They too are an

[31] Smith, E. V. 1920. The taking of immature salmon in the waters of the State of Washington. Washington Department of Fisheries, Seattle. / Pressey, R. T. 1953. The sport fishery for salmon in Puget Sound. Washington Department of Fisheries Research Papers 1:33–48. / Haw, F., H. O. Wendler, and G. Deschamps. 1967. Development of Washington State salmon sport fishery through 1964. Washington Department of Fisheries Research Bulletin 7.

[32] Chamberlin, J., A. N. Kagley, K. L. Fresh, T. P. Quinn. 2011. Movements of yearling Chinook salmon during the first summer in marine waters of Hood Canal, Washington. Transactions of the American Fisheries Society, 140(2):429-439.

trout			ESA Threatened species, whose abundance has declined. Coastal-PS DPS listed in 1999. Probably a more important predator in freshwater.
Resident Chinook salmon	SA, OP	Decrease	No evidence that resident Chinook salmon eat steelhead. Resident Chinook tend to be bottom oriented and steelhead likely surface oriented. Resident Chinook do consume smaller Chinook salmon (Beauchamp and Duffy 2011, PSC report)
Spiny dogfish	SA, OP	Decrease	Opportunistic, known to eat salmon, herring and other species of similar size to steelhead smolts, but primarily feed on invertebrates (Sturdevant et al. 2012). Can respond to high densities of hatchery smolts (Beamish et al. (1992)
Salmon sharks	SA	?	Do eat salmon in the ocean, likely low abundance in PS (Sturdevant et al. (2012)
Pacific hake, cod, and walleye pollock	OP	Decrease	These groundfish species were petitioned for listing under ESA (determined not warranted). Pacific cod declines concurrent with steelhead abundance and SAR declines. These groundfish species probably spatially (i.e., vertically) isolated from steelhead smolts
Lingcod	SA, OP	Decrease	Consumption of salmon typically for larger (>70 mm) lingcod, and up to 13% of diet by weight can be salmonids (Beaudreau et al. 2009). No evidence of steelhead predation. Spatially (vertically) segregated to some degree. S. Puget Sound populations more depleted than north Puget Sound

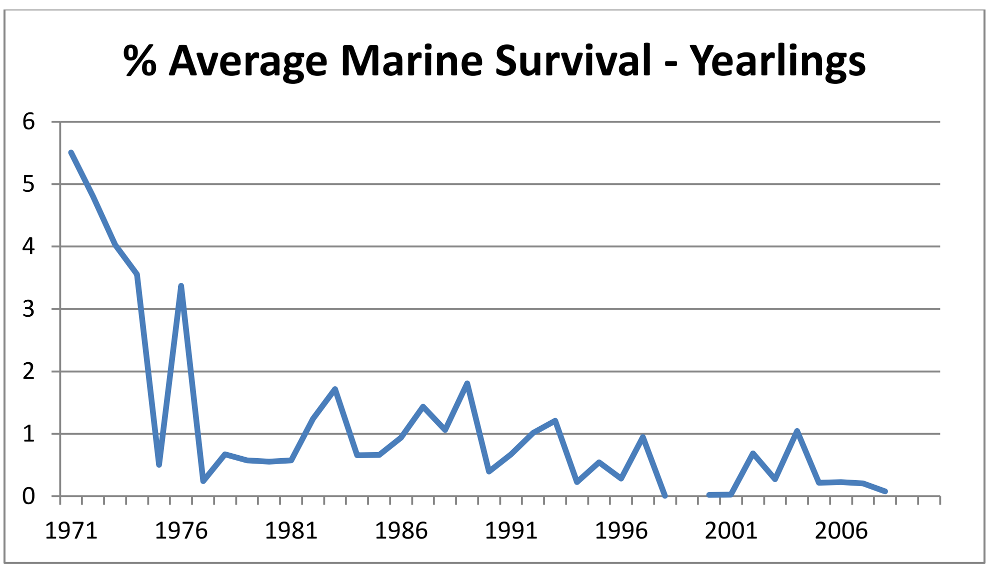

Figure 24. The average % smolt-to-adult survival of yearling hatchery Chinook produced throughout Puget Sound, 1971-2008. Based upon data from the Regional Mark Information

System database (contact Long Live the Kings for details regarding specific populations used to establish the average)

3. We suggest the SRWP include a definition of direct/proximate consistent with the Workgroup's use of the term.

   Response: *The report has been edited to address this comment.*

2) Jeff Hard (NOAA Northwest Fisheries Science Center and Puget Sound Steelhead Technical Review Team)

4. "…distinct patterns of mortality were observed among populations from the same region." Do you mean from different regions? AND How do the rapid travel rates narrow the list of potential mechanisms causing mortality in Puget Sound (specifically, which mechanisms are far less likely)?

   Response: *Distinct patterns of mortality were observed in different regions, yes. The report has been modified accordingly. Sections 1 and 2.1 of the report lay out the description of rapid travel rates in the context of some of what we know about outmigrant behavior and mortality, and the behavior and mortality patterns of steelhead are referenced in greater detail in the sections describing each potential cause of mortality. The actual ranking in Figure 1 was a loose interpretation based upon how potential factors contributing to mortality align with what we know about steelhead as they migrate through Puget Sound.*

5. It appears that summer-run fish in Puget Sound are not considered in the plan (e.g., Tolt River or Deer Creek). The data from B.C. (e.g., Figures 7 and 10) don't shed much light on whether these fish are likely to do anything differently from winter-run fish during early marine migration, or not. I think this would be an interesting component to consider adding to the design, especially if the workgroup could tap into existing monitoring.

   Response: *We will consider summer-run Puget Sound fish in our analyses. Specifically, we have data from both summer- and winter-run hatchery steelhead. Summer vs. winter run will also be considered as a factor in our analyses. Data from wild summer-run fish do not exist, though.*

6. 2.1, p. 12: A) How have they accounted for lack of detection as a source of "mortality" within Puget Sound from the acoustic telemetry work? B) To what extent might the low SARs be due to mortality outside Puget Sound? (How much does this really matter?)

   Response: *A) The model we will use to estimate survival of steelhead smolts also estimates the detection rate of each telemetry array, so lack of detection will not be attributed to mortality. Rather, detection rate will be used to adjust the number of smolts encountering each array to produce the final survival estimate. B) We don't think that the low SARs are primarily due to mortality outside of Puget Sound (i.e., open ocean), because coastal and Columbia River steelhead populations have experienced recent increases in SARs while Puget Sound SARs have* *declined (see Figure 3 of Research Work Plan). Unless Puget Sound steelhead migrate to different marine waters than do coastal and Columbia River steelhead populations, we would see similar declines in all Washington population SARs if open ocean mortality were the primary driver.*

7. 3.2.1-5, p. 13-19: A concise, well-documented, and informative review of the potential influence of predators, alternative prey, and other factors on steelhead marine survival in the Salish Sea. With regard to future tagging and monitoring work on harbor seals in particular, this seems important. However, key details about the design of the work, especially its spatial and temporal aspects, and how it would be used with bioenergetics and ecosystem modeling are lacking, and therefore it is difficult to evaluate their likely effectiveness.

   Response: *A more complete version of the harbor seal predation study has been provided in the final version of the plan.*

8. 3.3, p. 19-22: I thought this section was a logical way to bring the subject of poor condition and behavior into the discussion as a hypothesis to test. What is the evidence that potential predators typically target schooling species (versus that they are simply opportunistic foragers)?

   Response: *The evidence is based in part on what species marine mammals target but also where they target them. For example, seals and sea lions congregate at river mouths to capture returning adult salmon (pers. comm. Jeffries, Washington Department of Fish and Wildlife, 2013), and sea lions are known to target prey when nearshore and densely schooled in spawning aggregations or highly concentrated migratory movements (Sinclair and Zeppelin 2002, complete reference in the report). However, marine mammals are also known to be opportunistic.*

9. Appendix 3: Complete Descriptions of the Research Components: This appendix is very helpful because the hypotheses to be tested are clearly articulated and the general approach to testing them is briefly described. Nevertheless, it is still difficult for me to evaluate some of the study designs for their likely efficacy and power because key elements of the designs are often absent. As one example (Study 2: Assessing existing SAR data for trends), under "Study design" the document simply states that "these SAR data will be statistically analyzed and correlated with various steelhead individual and population characteristics along with Puget Sound environmental data, in part to account for variability in the estimates." What methods will be used to evaluate the trends and correlations, and how will fixed factors such as watershed or subregion be incorporated into the analysis?

   Response: *More complete work plans have been added to the report.*

10. Regarding Study 6 (Genome-wide association study of acoustically tagged steelhead smolts in the Salish Sea: measuring differences between survivors and non-survivors), this should be a very interesting analysis. However, the description mentions only that "we will use a variety of models to test statistically for a relationship between mapped SNPs and smolt survival." Presumably these would involve general linear models that include variables such as population and year as cofactors (e.g., Hecht et al. 2013), but what about considering a formal survival analysis (using, e.g., a Cox regression framework for censored survival data)? See, for example, Lin et al. (2011): Kernel machine SNP-set analysis for censored survival outcomes in genome-wide association studies. Genet. Epidem. 35:620-631.

Response: *Ken Warheit (WDFW), the principal investigator, agrees with this statement and will consider these methods for his analyses. The report has been modified based upon your comment.*

11. The description for Study 7 (Conduct a Puget Sound-wide fish health assessment of outmigrating steelhead) provides what I consider to be an adequate level of essential detail for a proposed design, though the details of the statistical analysis are absent (however, this would entail a fairly conventional analysis).

    Response: *The report has been modified accordingly.*

12. Regarding Study 8 (Perform a reciprocal transplant study to partition population effects from Puget Sound location effects), this is a focal point of the overall research and its results are key to helping resolve where early steelhead mortality in Puget Sound is occurring. The design appears to be robust, given the budgetary and logistical constraints. The power to test the stated hypothesis is, however, not known precisely.

    Response: *The report has been modified accordingly.*

13. One topic that does not appear to be considered in this work plan, but worth considering in future is an evaluation of the influence of SAR on iteroparity, and the consequent influence of iteroparity on productivity. This would require careful monitoring using genetic and/or tagging methods on a tractable system(s), but this could be very fruitful. Some exploratory, unpublished modeling suggests that the influence of iteroparity on productivity could be substantial, and the decline in iteroparity under reduced marine survival is a strong trend. Some of the patterns in Figures 3-8 may be heavily influenced by declining iteroparity, but this remains an open question generally.

    Response: *Barry Berejikian (NOAA) and Jeff Hard's email conversation in response to this comment is captured as follows:*

    Barry *On the issue of iteroparity……do you see a connection between SAR or early marine survival (i.e., smolts migrating through Puget Sound) and iteroparity? We discussed how iteroparity can influence productivity through increases reproductive success of females, but didn't see a direct link to the marine survival issue, unless of course we broaden the scope of the project to include kelt to adult survival (KARs?). Would you be willing to expand a bit on your comments (I've pasted them below), or clarify if you were referring to KAR (now it's an official acronym) or expand if you see a link between SAR and iteroparity. Thanks again!!*

    Jeff *I shouldn't have used SAR in my comment and got a bit off track there. I was in fact considering marine survival more generally when thinking about steelhead productivity. So you are right—when I was mulling this issue over I thought that ideally it should include consideration of kelt survival as well. I realize that this is probably outside the scope of the proposed work, but the modeling and little bit of data that we have seen does suggest that increasing the rate of iteroparity and survival of kelts could be critical to improving the abundance and productivity of steelhead in Puget Sound, especially in years when ocean conditions are poor. I imagine that the high abundance of harbor* *seals, porpoises, etc., could really take its toll on kelt survival. Sorry for the confusion. Hope this helps to clarify my comments.*

14. Finally, with regard to the proposed modeling (either ecosystem, or bioenergetics), there is almost no information provided on the approach to be taken in Study 11, other than mentioning some candidate models (e.g., Ecopath or Ecosim for bioenergetics). The same is true for Study 10 to test the 'dinner bell' effect. Additionally, one type of modeling that I think would be very valuable to add to those proposed would be some demographic modeling of population dynamics, parameterized as far as possible with realistic values of age-specific survival and fecundity data. This could incorporate conventional age- or stage-based population dynamics models, or perhaps a novel Integral Projection Model (Coulson, T. 2012. Integral projections models, their construction and use in posing hypotheses in ecology. Oikos 121:1337-1350). Food for thought.

    Response: *Details about investigating the dinner bell hypothesis are embedded in Study 8: reciprocal transplant description. More detail has been added to this study description. Details regarding ecosystem/food web modeling referenced in study 11 will be provided in the forthcoming Puget Sound Research Plan for multiple salmonid species, being completed by the U.S. Salish Sea Technical Team for the Salish Sea Marine Survival Project. However, note that the group has not put much weight in these models for evaluating steelhead impacts due to the very small abundance of juvenile steelhead relative to other fish species that comprise specific trophic levels of the food web. We will consider including population dynamics demographic models, such as Integral Projection Models.*

3) John Kocik (NOAA Northeast Fisheries Science Center)

15. The potential for sub-lethal fw challenges to manifest themselves in the ocean as mortality is real and might warrant mention - even if you yellow light it! Check out :

    Fairchild, W. L., Swansburg, E. O., Arsenault, J. T., & Brown, S. B. (1999). Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (Salmo salar) represent a case of endocrine disruption?. *Environmental Health Perspectives*, *107*(5), 349.

    McCormick, S. D., O'Dea, M. F., Moeckel, A. M., Lerner, D. T., & Björnsson, B. T. (2005). Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17β-estradiol. *General and comparative endocrinology*, *142*(3), 280-288.

    The scary thing is that it is the delivery system not the pesticide in Fairchild's work!

    Response: *Sub lethal effects that may manifest themselves in freshwater are being evaluated via the reciprocal transplant and fish health assessment studies. Endocrine disruption was specifically discussed when a broader group of scientists was brought in to determine the focus of the fish health assessment. Penny Swanson (NOAA) concluded that that the telemetry data* *are not consistent with physiological issues such as stunting and parr reversion. If that were the case, one would expect the steelhead to delay entry into the marine environment or return to the freshwater. One also may expect to see size-selective mortality occurring, with smaller, less developed fish performing more poorly. The group then concluded that investigating physiological issues (Transcriptome analysis, hormone measurement) will not be a priority for this analysis. That said, the juvenile steelhead will be analyzed for the presence of toxic chemicals from the freshwater out through the marine environment via the fish health assessment (study 7).*

16. My only major comment related to the overall project gains is better formalizing how you or some team leader is going to integrate and synthesis all 11 studies in a team framework. Maybe it is in the proposal and I am missing it but calling it out as its own study would give the metaproject higher impact and gains- and give you a dozen.

    Response: *The following has been added to the Project Coordination section of the report: " The results of all the studies in the work plan will be comprehensively evaluated by the Workgroup as a whole, and will be presented to outside experts in aggregate for review and discussion. This will be led by the Washington Department of Fish and Wildlife project manager, Neala Kendall, and project coordinator, Michael Schmidt of Long Live the Kings. This includes hosting sessions at conferences or workshops such as the biennial Pacific steelhead management meeting hosted by the Pacific States Marine Fisheries Commission. A series of workshops will be held to disseminate the results and discuss them in aggregate under the umbrella effort, the Salish Sea Marine Project. "*

17. Figure 1 – seems like climate change should be explicit in Q3 panel – or at least highlighted under habitat modifications

    Response: *The following was added to the "Overview" section of the report. "Ultimately, an additional step beyond this research is to determine whether the factors affecting survival are fundamentally the result of local human-induced pressures (e.g., habitat change, toxics, species management) or larger shifts (e.g., from climate and ocean variation, temperature and OA) differentially affecting an inland sea (when compared to the coast). For example, larger-scale habitat changes, such as temperature regime shifts associated with climate change or ocean cycles, could fundamentally be affecting the prevalence of disease, presence and abundance of buffer prey, changes to turbidity, and changes to marine predator migration and residence patterns."*

18. Study 9 Do you have access to hatchery or sea-run kelts? If so, I strongly encourage their use in this evaluation. I would still support use of smolts but suggest adding kelts would be a multiplier to study utility. Atlantic salmon information suggests that kelts are reasonable proxies for virgin returning adults. We have short absence returns so kelts would not only give you outmigration data but potentially adult return data. This is worth thinking about.

    Response: *Natural kelts are extremely difficult to access, and the focus of this work is on juvenile outmigrant mortality based largely on the acoustic telemetry results showing high early marine mortality, suggesting that is where to focus.*

19. Study 10. We are curious about this too but less for salmon then for sturgeon. One thing to remember is that tags that end up in scat or natural morts that end up in the water – as such there are dinner bells with no food at the end of the sound. This brings up a point. Are there active searches (boats or AUV's) using acoustic receivers? We are able to locate many of our tags and from location – cormorant rookery or seal haul out and then generate Ho as to predator. Also, individually-based analyses of behavior is important; if tags fly over detection units or swim at 40 body-lengths per second, your tags are no longer in steelhead. A pass-fail survival analysis will miss this. Finally, I strongly suggest you incorporate random search transects and targeted predator location monitoring as the qualitative benefits in information greatly outweigh costs.

    Response: *All good ideas; however, these additions would exceed the capacity of the scientists doing this work. The location of the harbor seals is being monitored with GPS. Mobile tracking has been attempted in previous experiments and it is extremely labor intensive and time consuming, especially since we don't know where deposited tags might be and because the smolts are moving rapidly. That said, if hotspots can be isolated in year one, follow-up studies may incorporate mobile tracking.*

20. Controlled experiments – suggest exposure studies have a saltwater challenge element to them

    Response: *While no controlled experiment was initially included as part of the fish health assessment. . The intended approach in this phase of the project is to determine whether field surveillances of pathogen prevalence / intensity support the hypothesis of pathogen-associated mortality. If the field results do not support this hypothesis, then the disease hypothesis will be dismissed. However, if the field surveillances do support the pathogen-associated mortality hypothesis, then additional controlled studies will be recommended. The Workgroup initially decided not to combine the field and laboratory studies in the same year because:*

    1) The information obtained from the field studies may negate the need for the controlled studies

    2) Cost

    However, concerns about the success rate of capturing steelhead in the estuary and marine environments have resulted in a change to the fish health study. The study will now include a component that tests in lab the outcome of nanopheytus infections when steelhead are in the marine environment. Steelhead with different levels of nanopheytus exposure will be trapped in the freshwater and then transferred to a laboratory setting for evaluation. This was added as a backup due to as proposed in the fish health assessment. This component will include a sweater challenge.

21. Figure 23 and in text – is cleaner water result of top down control of phytoplankton (trophic cascade) or bottom-up phosphorous/nitrogen reduction. Driver may be important to understanding ecological impacts.

    Response: *There is evidence that both drivers may be occurring. Christopher Krembs of the Washington Department of Ecology has been investigating this. The Puget Sound Steelhead Marine Survival Workgroup and the Technical Team of the Salish Sea Marine Survival Project will* *be following the progress of his efforts and incorporating results into broader thinking about what is fundamentally driving the survival of salmon and steelhead in Puget Sound.*

4) Sean Hayes (NOAA Southwest Fisheries Science Center)

22. Less of a comment, and more of an emphasis of how important it is that the researchers test the 'dinner bell hypothesis' with as much rigor as possible. Given recent observations both referenced in the document and other papers now in review (Cunningham et al. in review), it is clear that 69 kHz tags are clearly audible to harbor seals from significant distances. As it is these acoustic tracking mortality that places the target area of focal research on central Puget, it is absolutely critical to confirm that the mortality rates observed by Moore et al are not biased by seals simply being able to target tagged fish. It is only once this concern is ruled out can the full experimental design of all the other studies be realized, and researchers move forward for confidence that they are looking for mortality in the appropriate location and life stage. (on that note- I would strongly recommend some power analysis about adequate sample size needed to confirm/refute this hypothesis (if its not already done).

    Response: *Barry Berejikian, the Principal Investigator of this particular study, agrees. He states, "A preliminary power analysis suggests that we should be able to detect a doubling of the typical survival rate (20% pinging group) to 40% (non-pinging group) with 1-B = 0.80 and alpha = 0.05 at the sample sizes indicated."*

23. While I suspect the proposed studies probably provide adequate scrutiny, my own research team has been recently surprised at the amount of freshwater mortality that is happening both during freshwater migration stages of hatchery released fish, as well as immediately downstream of smolt trap stations. In some cases mortality rates as high as 96% are observed in watersheds ranging in size from the Sacramento basin (Michel 2010, Hayes et al. 2012) to just the last few hundred meters of small coastal basins and prior to final ocean entry (Frechette et al. 2013, Osterback et al. 2013). Some 'back of the envelope estimates' compensating for freshwater mortality in stocks with SARS of <1%, suggest marine survival for many of these stocks could be 15-20 percent for steelhead and subyearling chinook alike (Hayes et al. 2013). In our own naiveté, for years, we overlooked the potential impact of a few common mergansers, or the unsuspected impact of western gulls at small coastal river mouths, or large introduced piscine predators in the central valley simply because 'salmonids were not observed' in diet. In the review process proposed for Study 5, I would recommend taking a much closer look at seemingly anecdotal observations and presumptions about predatory abilities of a candidate species to have an impact- particularly at the shallower estuarine freshwater interface (I saw Jeffries comment about absence of evidence here but provide follow up comment in document). Also- none were mentioned.. but are there any new piscine predators (a huge issue in CA- bass etc)? And or any shifts in predator salmonid populations? like can cutthroat populations impact steelhead smolts at all? Any new hatchery stocks of resident Chinook that might be piscivorous on steelhead?

    Response: *We just don't see evidence high mortality in the river mouths/estuaries within Hood Canal or Puget Sound. One exception may be the Green/Duwamish. I think there's a real difference in the coastal vs Puget Sound estuaries. Results from the Oregon coast concur with* *your findings, but appears much different in PS. We typically see high downstream migratory survival in the telemetry studies, with the exception of some hatchery stocks that may also include some residualism. But at our next meeting I'll make sure we bring this up to make sure we're not missing something. Also see the response to comment 2, above.*

24. The convergent goals of studies 4 and 8-11 all seek to address the potential impact of harbor seals and other predators on juvenile salmonids. Several points for emphasis and I apologize if they are seemingly redundant with what is already proposed, but bear with my thought process (or don't ;-)) A question that needs to be asked is- given the 4-5 fold increase in harbor seal populations in PS, while it clear that steelhead populations are too small to support that, one must ask the question- What are the seals eating? And how is that affecting the food web as a whole- the authors propose things like relating steelhead survival to variables like herring population densities… but be sure to consider what the actual buffering potential of a 20-30% change of herring populations might have if there is a 200% increase in predator demand?

    Response: *The Workgroup agrees and is working to evaluate both the potential reduction in buffer prey (e.g., Cherry Point herring) and increase in predator populations. Study 4 attempts to get at this, as well as reciprocal transplant and harbor seal encounter studies. A comprehensive evaluation of the cumulative effect of multiple factors may take place more rigorously via food web modeling once more data are compiled. This is addressed in greater detail in the forthcoming Puget Sound Research Plan for multiple salmonid species, being completed by the U.S. Salish Sea Technical Team for the Salish Sea Marine Survival Project.*

25. Further on the theme of #3… the authors rightly surmise that harbor seals are likely to have an impact even if steelhead are a small part of their diet given their energetic requirements and population size. However, they express concerns about being able to quantify this statistically given the relatively low proportion of habor seal diet steelhead would likely represent. Just because something is rarely observed does not mean its not happening at a significant level (Estes et al. 1998) In essence I suspect they are concerned about statistical power to test between an diet contribution of 2 vs 0.1%. But perhaps they might want to reconsider the question from the opposite direction- specifically.. ask two questions:
    a. what proportion of the harbor seal population diet would steelhead have to be to account for it to have a population level effect?
    b. What are the odds that a steelhead would be detected in a scan of 100, 1000, 10,000 scat samples at that level? Just running some simple back-of-the-envelope estimates.. if 10,000 harbor seals each consumed 2kg fish/day for 90 days (smolt migration season) (Howard 2009) that would be 1.8 million kg of fish (you gotta wonder what it all is…).. assuming average smolt size of ~160mm/45g (?).. a hypothetical range of diet percentages is tabled below.. even if steelhead were only .1% of harbor seal diet.. there would be 40,000 missing smolts in this scenario. It would be worth getting a bioenergetics expert, a diet analyst and a statistician together to ask- what are the odds

of detecting that if it were far less frequent?

% steelhead in diet	# 45g smolts consumed
100%	40,000,000
1%	400,000
0.1%	40,000
0.01%	4,000

Response: *Barry Berejikian performed the same calculations and concluded that without some knowledge of where to look, approaching the harbor seal question through diet analysis is a needle in a haystack. Seals in northern Puget Sound eat alot of pollock, herring and sand lance, but also have a fairly frequency of occurrence of unidentified salmonids (32% in July Thomas et al. 2011 Mar. Ecol. Prog. Ser.), so there may be some opportunity to figure out what type of salmon they're consuming. However, as you showed, it would be a stretch to find steelhead even if a high % of them are consumed by seals. Austin Thomas of the University of British Columbia, along with others, is proposing a more comprehensive analysis of harbor seal diets in the Salish Sea. We are working to coordinate with him to the greatest extent practicable to see if they produce any results related to steelhead.*

26. A final area of further consideration which I think it touched upon by study 2 and 3. The brief descriptions focus on smolt size numbers and in the appendix briefly mention timing in outmigration. I see that timing of outmigration is also mentioned in the appendix.. but I would be curious about the potential for shifting climate trends to be creating a disconnect timing of outmigration and timing optimal ocean conditions and that the two could be getting pushed in divergent directions. An interesting paper by Kock et al (Kock et al. 2012) considers how watersheds transitioning from snow melt to winter runoff could result in timing of coho smolt migration.. and a recently accepted paper by Friedland et al (Friedland et al. accepted with revisions) (copy attached) places new light on how ocean conditions may relate steelhead smolt survival on the Keogh. This final one.. perhaps moves the focus away from central Puget Sound mortality issues… not never the less is potentially quite significant. Also- I would emphasis that there is probably no single smoking gun here and rather populations (clearly even coastal ones based upon your figures 3 & 4) are declining impacts of multiple variables…. Assessing shifts in migration timing patterns and assessing ocean conditions are two big (but the latter – a bit nebulous) issues that need to be explored.

    Response: *As you state, this will be addressed in Study 2 and 3 by looking at outmigration timing over time, comparing Puget Sound to coastal populations, and comparing populations with better vs. worse smolt-to-adult survival rates. Barry Berejikian also states: "We have struggled to estimate whether outmigration timing has or will shift with climate change. Best I can think to do is quantify outmigrant timing of rain driven systems vs transitional systems (with a second peak from snowmelt). We see pretty strong structuring with warmer, rain dominant systems producing earlier outmigrants (about 2 weeks). So we might use that to predict future migration timing for transitional systems. So far, we've not been able to detect timing effects on early marine survival, but I'm not sure we've dug deeply enough into the telemetry data yet."*

5) Puget Sound Steelhead and Cutthroat Policy Advisory Group (SCPAG)

The Puget Sound Steelhead Marine Survival Workgroup and SCPAG agreed that the notes resulting from their October 30th 2013 meeting would suffice as responses to their comments. The notes are as follows:

Salish Sea Marine Survival Project
PUGET SOUND STEELHEAD STAKEHOLDER MEETING NOTES

October 30, 2013 (12:30-2 pm)
NRB 630
Olympia, WA

Attendees: Sandie O'Neill, Barry Berejikian, Steve Jeffries, Ken Warheit, John Kerwin, Paul Hershberger, Michael Schmidt, Jim McRoberts, Pete Soverel, Hal Boynton, Al Senyohl, Frank Urabeck, Scott Pearson, Erik Neatherlin, Neala Kendall, Iris Kemp

Meeting Objectives: Discuss steelhead work plan with interested stakeholders.

Action Items

- The Steelhead Workgroup will provide stakeholders with these compiled meeting notes to reflect the discussion and response to stakeholder comments.
- Erik N. and Michael S. plan to present a short update at the Steelhead and Cutthroat Policy Advisory Group (SCPAG) meeting in February, and a full update at the June meeting. Workgroup members will be brought in to do presentations/discussions when appropriate or requested by the SCPAG.
- The Workgroup will work with the SCPAG to advocate for folks in the region to do representatively acoustic tag more steelhead populations in 2014.
- The Workgroup will, to the extent practicable, compare survival of populations of North Sound sea-run cutthroat and bull trout populations, and moreso of Deer Creek and Coquihalla summer steelhead runs, that are generally considered healthy, to Puget Sound steelhead smolt-to-adult survival trends.
- Pete Soverel will send Michael S. relevant contacts in the Ministry of Environment, B.C.

Notes

The stakeholders initial comments to the draft steelhead work plan were to: 1) incorporate a mini-barging study, 2) identify management responses, 3) address possible predators other than harbor seals and porpoises, and 4) ensure potentially, comparatively healthy populations of steelhead and cutthroat and bull trout are considered to generate more context for isolating where and how mortality is occurring.

Barging Study

- The Salish Sea Marine Survival Project's Steelhead Workgroup has discussed several barging study designs at length, but ultimately decided against their inclusion for several factors.

- Based on 3-4 years of acoustic telemetry data, which showed that steelhead outmigrate in 1-2 weeks. The only factor that the group can think of to cause such rapid mortality is predation. The question is whether the fish that get eaten are healthy or compromised.
  - **Hal B.** is concerned about the reliability and detection range of the West Point line, and any study based on it. What if different populations use different migratory pathways? **Barry B.** feels confident about their estimates; mark-recapture models account for the efficiency of the line. If different populations migrate differently, it might violate the model assumption of equal group detection. The study we have proposed will answer that question.
  - Additional value from the proposed acoustic telemetry plan is creating a receiver network infrastructure that will remain in place 5-7 years into the future.
  - The stakeholders suggest increased communication with tribal groups and other groups who may be interested in tagging fish (e.g., Puyallup and Blake Smith).
- **Barry B.** says that a barging study is based on a one-tailed hypothesis: if the barged fish survive at a higher rate, then you learn something (the water didn't kill them, predators didn't kill them); but, if the barged fish do not survive at a higher rate, you learn nothing (confounded by potential barging effects). The potential knowledge gained from the plan we put together to understand compromised fish health and locations of mortality outweighs potential knowledge from a barging study. **John K.** adds that we have very little experience with saltwater barging.
- **Neala K.** comments that we also had to bear in mind what management actions could be taken based on the results of the study. If barging increases survival, should we start barging en masse and introducing imprinting issues? Our plan is intended to localize the issue points.
- **Erik N.** says the group was looking for foundational studies that could anchor the research. Retrospective work to make sure we understand how we got to this point was easy to identify. We talked for a long time about a barging study as a pathway to anchor the research plan, but decided against it because we are already assuming something is happening to the fish and we can't think of anything other than predation that happens over such a short timeframe. The barging study is not a tenable base.
- **Barry B.** has a paper from BC on barging fish past hotspots to increase survival. He will send it to the group. However, we are still trying to figure out if/where the hotspots in Puget Sound are.

Management Responses

- The Steelhead Workgroup thinks that the approach they proposed is important towards management issues because we are identifying specific problems and trying to understand the root cause of mortality. For example, perhaps there is a bird species targeting steelhead, but the fish are only susceptible because they are sick and float near the surface. In that case, management actions to reduce the bird population would not solve the steelhead problem.
- **Frank U.** asks whether we will be able to take management actions by the end of this year. How soon before we can save fish?
  - **Barry B.** says this group is trying to identify the core reasons that steelhead are dying in Puget Sound, and we are doing it as fast as we can. We cannot tell you we will have management actions by a specific date, but we can tell you that we believe our actions push the ball as far forward as it can get right now.

  - **Erik N.** asks how we could know what action to take without understanding the root causes. Our proposed plan will address identification of root causes: for example, we will be able to say whether there is freshwater contribution to marine mortality.

Potential Predators

- **Scott P.** explains that his study plan considers several potential predators and narrows the list down to the most likely based on a variety of questions: who eats salmon, who eats steelhead, who feeds at steelhead migration depth and location, who targets steelhead-sized prey, who is abundant in Puget Sound during the steelhead outmigration period? Additionally he is looking at trends for predator populations (abundance and/or distribution). It's difficult to identify with 100% certainty any one specific predator species, particularly with the low biomass of steelhead. There is some potential with scat analysis and new PIT-tagging technology in development at UBC.
- If we can identify a predator, are we willing to do something about it? Potential options are reducing predator populations or supporting buffer prey populations.
- **Hal B.** asks if the harbor seal interaction study is still included.
  - **Barry B.** responds that it is. Steelhead from the reciprocal transplant study are subjects for the interaction. We will outfit seals from Blakeley with tag packages that take GPS data, can be detected by lines, and will detect other tags. We can calculate detection range of the seal with stationary sentinel tags. We chose Blakeley because by concentrating there we have good coverage of what we think is a hotspot.
  - **Pete S.** asks whether the seals can hear the pings. **Barry B.** says that the tagged seals will hear the pings all the time anyway. Also, hearing tags do not mean they will target tags. To test the dinner bell hypothesis (that seals target fish with tags), we will have a subsample of tags that are silent and begin pinging after the fish travels through Central Sound. We intend for each river (Green and Nisqually) to have 60 normally-tagged fish, 10 silent-tagged fish, and 60 normally-tagged fish transplanted to the reciprocal river. So that is 260 tagged fish in total.
    - **Hal B.** is concerned that we will have logistical problems with flow and that the Corps may be willing to assist. It is important that we get enough fish. **Michael S.** says those conversations are happening now.

Other

- **Pete S.** comments that not all systems are equally bad; we should try to understand regional differences in survival. Also, sea-run cutthroat trout and bull trout have similar nearshore marine life histories and are both in good shape. What is the difference between these species and steelhead? Finally, Deer Creek and Coquihalla summer runs of steelhead are doing pretty well. If the problem is predators or disease, why are these stocks not impacted?
  - **Barry B.** states that they have tracked Hood Canal steelhead, cutthroat, and cutthroat-steelhead hybrids. Steelhead migrate out quickly and in the center of the channel, but cutthroat migrate nearshore and hybrids are somewhere in between. The behaviors of each are very different.
  - **Paul H.** says that often some species are more susceptible to certain diseases than others, so disease can have different impacts by species. *Nanophyetus* is carried by a snail intermediate host which is primarily found in South Puget Sound, so that may explain some geographical differences. It also affects coho and stretches down the coast. **Barry B.** notes that when comparing abundance over years, the severity of decline appears as a gradient,

from North (bad) to South (worse). When considering hatchery effects and that Nisqually (a reference water quality stream) does not have hatchery releases, it suggests that something is going on in the Central basin.

- **Al S.** fishes through the season and has noticed that smaller salmon smolts seem prevalent, and assumes sockeye also. It is odd that sockeye are doing okay but steelhead are not. Could migratory timing have something to do with it? For example, if steelhead outmigrate at a time when there are no other prey items for predators to eat?
  - The group agrees that this is a good question – we are wondering these things too.
- **Pete S.** stresses the need to coordinate with British Columbia, because steelhead stocks are in trouble throughout the Salish Sea.
  - **Michael S.** reports that Brian Riddell, with the Pacific Salmon Foundation, has been supportive of including steelhead in their plans, but hasn't yet considered how to do it. The Canadians have been focused on Chinook and coho. **Pete S.** says he has no faith in DFO Canada regarding steelhead and that we should contact the BC Ministry of Environment. **Pete** will send contact information and **Michael** will talk to Brian.
- **Michael S.** outlines the group's projected progress:
  - We will schedule 6-month progress reporting intervals from June 2014 to June 2015. We can increase the frequency of reports towards the end of that time period if there is interest. Most studies will produce manuscripts.
  - Most of the retrospective work with data compilation and wild/hatchery comparisons is complete. Next we will begin looking for correlations. Those preliminary results will be available June 2014.
  - Results from the GWAs study will be available January 2015. Initial reports from fieldwork will also happen at this time.
  - **We agree** that we will attend steelhead/cutthroat advisory meetings to provide updates: a short update in February and a full update in June 2014. We will provide Powerpoint presentations and answer questions.
- Cost for the dinner bell study is not broken out because it is nominal.
- The modeling study is included in case there is opportunity to take advantage of modeling efforts developed via other Salish Sea Marine Survival Project and partner activities; state appropriated funds will not be used for this. It is a lower priority because of Puget Sound steelhead's relatively small role in the Puget Sound food web (hard to get meaningful results from ecosystem modeling when this is the case).