Saltonstall-Kennedy Grant Program

Ecological, Genetic and Productivity Consequences of Interactions between Hatchery and Natural-Origin Steelhead of the Skagit Watershed

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1.0 General Introduction

Puget Sound steelhead (*Oncorhynchus mykiss*) were listed by NOAA as Threatened under the Endangered Species Act in May of 2007. A species listed as "threatened" is likely to become endangered within the foreseeable future. Puget Sound steelhead are defined as a "Distinct Population Segment" (DPS) and the ESA listing covers naturally spawned (wild born) steelhead from river basins draining to Puget Sound, Hood Canal and the eastern half of the Strait of Juan de Fuca. The listed steelhead include more than 50 distinct populations of summer- and winter-run fish. Most steelhead are found in northern Puget Sound areas where the Skagit and Snohomish rivers support the largest populations. Most of the hatchery stocks used in the Puget Sound DPS area were not included in the ESA listing because they either originated outside the DPS (Skamania summer-run stock) or they did not represent native local populations (Chambers Creek winter-run stock). Hatchery stocks originating recently from local wild broodstock steelhead were included in the listing.

It is thought that many factors collectively contribute to Puget Sound steelhead population declines by altering survival and productivity (Hard 2007). The Puget Sound Steelhead Technical Recovery Team (TRT) recently indentified the following factors of decline: the present or threatened destruction, modification, or curtailment of its habitat or range; overutilization for commercial, recreational, scientific, or educational purposes; disease or predation; the inadequacy of existing regulatory mechanisms; and other natural or manmade factors such as unfavorable ocean conditions and harmful hatchery practices (NMFS 2007).

Recognizing that many factors likely contribute to the decline of Skagit steelhead, this study's sole focus is on the potential effects of hatchery releases on natural-origin steelhead survival and abundance from an ecological and genetic standpoint. The findings of this report will be an important contribution to the development of a Skagit specific recovery plan that will address the full range of "factors of decline" and can be used by fish managers to form and support recovery decisions.

While it is difficult to monitor escapement and survival of steelhead, current literature suggests that significant genetic and ecological interactions occur between hatchery and natural-origin steelhead (Kostow et al. 2003; Mackey et al. 2001). It has been shown that even small contributions from segregated hatchery populations to small natural populations can lead to a significant loss of fitness (Lars Mobrand, 2005).

Kostow (2009) identified several factors capable of contributing to the ecological risks of steelhead hatchery programs such as; large releases of hatchery fish, hatchery fish increase density-dependant mortality, residual hatchery fish and physical advantages of hatchery juveniles. Other researchers have determined that hatchery steelhead can reduce survival rates of wild (and hatchery) steelhead through four mechanisms: habitat competition, direct predation, genetic interactions and disease.

Because of the challenges with quantifying introgression, seemingly the most direct measure of genetic impacts, we chose to take a weight-of-evidence approach in the form of a meta-analysis that incorporates a new methodology for quantification of introgression and natural spawning by hatchery adults in the wild, an evaluation of ancestry in juvenile and adult steelhead at the individual level, along with other evidence of hatchery adult straying and behavior traits at juvenile and adult life stages that are thought to contribute to interactions at both the ecological and genetic level.

Because natural-origin steelhead are ubiquitous throughout the year in Puget Sound and co-occur in fisheries on other species (spring chinook, sockeye), the take prohibitions on Puget Sound steelhead potentially restrict harvests on co-occurring non-listed species. Generally, winter steelhead tribal harvest management in the Skagit River watershed is directed primarily towards harvesting surplus hatchery and wild production during the early part of the run, then reducing harvest intensity when the wild run predominates, in order to achieve wild escapement objectives (HSRG, 2004). Similarly, the sport fishery is also directed at harvest of hatchery-origin steelhead with the requirement that any natural-origin steelhead encountered must be released unharmed.

In an effort to supplement harvest of steelhead, there are significant releases of non-ESA-listed hatchery steelhead throughout Puget Sound. These releases provide the basis for harvest of steelhead in Puget Sound. However, interactions between non-listed hatchery steelhead and listed wild steelhead have been identified as a factor causing the decline of wild steelhead; consequently, co-managers are considering the curtailment or elimination of many hatchery steelhead programs. This would effectively eliminate many tribal and sport steelhead fisheries. The potential impacts of these negative interactions between natural and hatchery-origin steelhead within the Skagit watershed had not been studied or documented. To preserve the future opportunity to harvest Skagit steelhead and co-occurring non-listed species it is imperative to examine whether and to what extent hatchery steelhead have been a factor causing the decline of wild steelhead, and what hatchery actions can be taken to address any negative interactions.

The fundamental goal of a segregated hatchery program like that used for winter steelhead in the Skagit basin is to propagate the hatchery broodstock as a discrete population or gene pool that is reproductively segregated from natural-origin spawning populations. Once established, a segregated broodstock is composed entirely of returning, hatchery-origin adults. Any natural spawning by hatchery-origin steelhead from a segregated program imposes a variety of potentially unacceptable risks to natural populations (Mobrand 2005).

As a foundation for hatchery reform, the Hatchery Scientific Review Group (HSRG; available at http://www.lltk.org/HRP.html) recommended that hatchery managers change their measure of success from the number of juvenile fish released, to the number of adult fish returning to sustain the stock and provide fishing opportunities. This means focusing on quality over quantity, understanding the carrying capacity of the freshwater and marine environments into which fish are released, and other scientifically sound hatchery practices. The development of scientifically defensible hatchery programs is critically dependent on monitoring the incidence of hatchery-origin juveniles and adults in natural spawning areas.

Hatchery reform fundamentally requires evaluating hatcheries based on how they affect the watershed in which they are located. This means a hatchery program—whether for harvest or conservation purposes—cannot be successful unless it serves the needs of the wild populations it is derived from and/or encounters outside the hatchery. The size of the hatchery program (i.e., number of fish released) must be considered in light of what the available habitat can sustain, and the habitat needs of other fish in the watershed.

With a segregated program hatchery fish are to be managed as a wholly separate population in which case strict guidelines must be met to ensure the hatchery fish do not influence or alter the wild population. In effect, the HSRG's guidelines ensure that the wild population is "in charge" of genetic and behavioral adaptation (HSRG, 2004). The 2004 HSRG report included a recommendation that an investigation be completed to determine reasons for the recent decline in adult winter steelhead returns to Puget Sound streams, formulate a working hypothesis for the decline, and take appropriate actions.

Management in the Skagit Basin is primarily directed towards the need of natural production; however, the steelhead hatchery program on the Skagit has a harvest-oriented purpose. The hatchery program uses a segregated strategy incorporating the Chambers Creek origin steelhead stock which has an earlier run-timing (due to selective breeding) than the Skagit basin wild winter-run steelhead stock (see Appendix 1).

Marblemount Hatchery, which is owned and operated by WDFW, is the only hatchery propagating steelhead smolts in the Skagit River. It is located near the town of Marblemount on the Cascade River. The hatchery complex also includes Barnaby Slough; a rearing pond for winter steelhead located at RM 70.2 on the Skagit River downstream of Marblemount Hatchery near the town of Rockport. The average 1995–2005 brood year (BY) releases from Marblemount hatchery complex were 413,900 with a maximum release in 2000 of 663,470 smolts and pre-smolts. The average BY releases from 1985–1995 were 286,337 smolts and pre-smolts. All fish are currently 100% marked with an adipose fin clip and released at 6 fish per pound in May to mimic the outmigration timing of wild smolt. Steelhead adult collection and spawning takes place from late December to March (see Appendix 1).

The primary goal of this study is to develop science-based management actions that will allow commercial and sport fishers to access harvestable fish while not impeding the recovery of wild steelhead stocks within the Skagit River Basin.

2.0 Study Approach

The approach to this study has three major elements. The first relied on the collection of genetic and biotelemetry data to directly evaluate interactions that may be occurring between hatchery and natural-origin steelhead conspecifics. The second element involves a genetically-based method of quantifying hatchery-natural-origin introgressive hybridization by first using an empirical model to define the statistical limits of the analysis, and then a quantitative assessment to determine the relative degree of introgression hybridization between hatchery and wild populations from the Skagit Basin. The final element utilizes existing smolt release and population trend data from three geographical zones; Skagit watershed, Puget Sound and regionally. This dataset was used to complete a statistical and descriptive analysis of hatchery smolt release impacts on natural-origin steelhead populations within the three geographical zones.

The findings from these study elements will collectively formulate a weight-of-evidence opinion that can be used by fish managers to decide whether hatchery program adjustments or elimination should be considered as part of a larger recovery planning effort. The ultimate goal is to achieve a level of recovery capable of sustaining some level of harvest.

2. 1 LifeHistory-based Meta-analysis

The study team chose to use a lifehistory based meta-analysis approach to help understand the genetic and ecological effects of interactions between hatchery and natural-origin steelhead during a variety of lifestage periods (Figure 1).

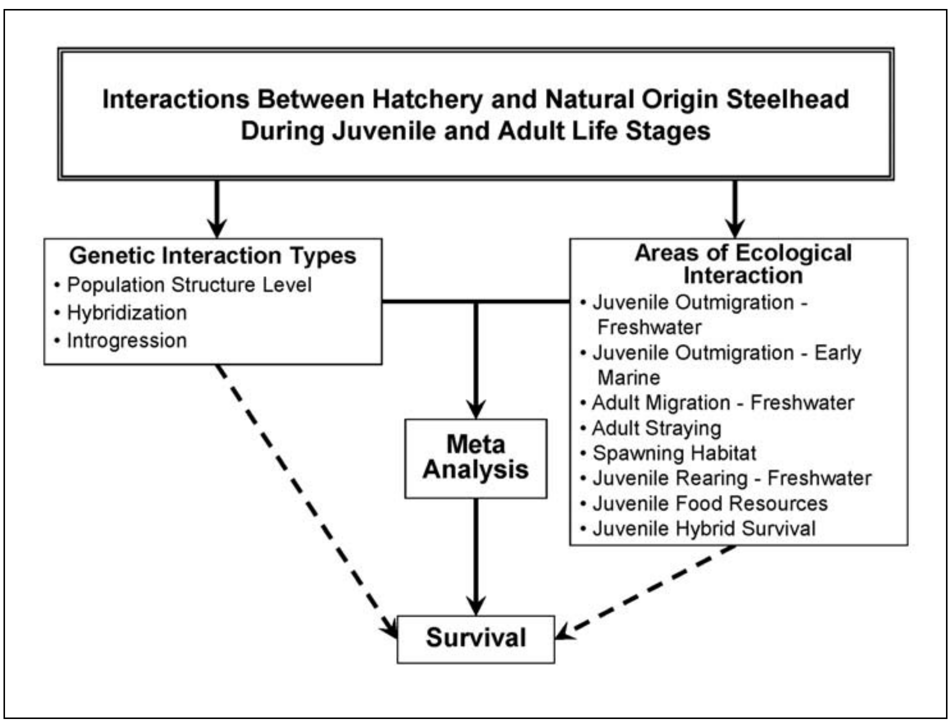


Figure 1. Meta-analysis diagram of hatchery and natural-origin steelhead interactions during juvenile and adult lifestages

The two major interaction factors used in this investigation were of a genetic and ecological nature. We studied the three most common lifehistory forms of O. Mykiss that are present in the Skagit; hatchery and natural-origin steelhead and resident form. We studied these lifehistory types at several juvenile to adult lifestage sub-periods by collecting genetic materials and by employing biotelemetry methods. Information was collected from within defined mainstem channel reaches or tributaries to evaluate possible spatial differences results (Figure 2).

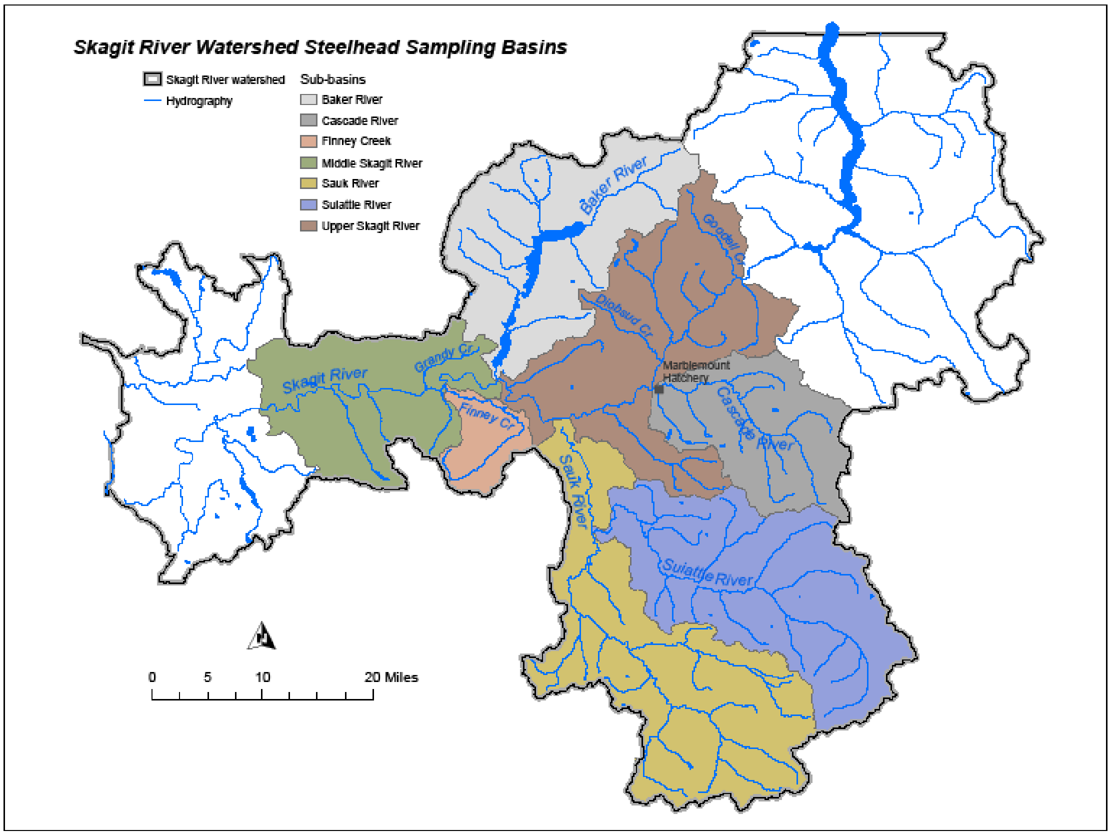


Figure 2. Skagit River study area showing important sub-basins and tributary streams (courtesy of Skagit River System Cooperative).

2. 2 Evaluation of Introgressive Hybridization

There are few tools available to quantify introgression in fish. For this reason, a new approach was developed to document the extent to which the segregated Marblemount Hatchery winter steelhead population has hybridized with the natural-origin steelhead within the Skagit River basin. The newly developed empirical model was used to define the statistical limits of the analysis in conjunction with a quantitative assessment to determine the relative degree of introgressive hybridization between hatchery and natural-origin juvenile and adult collections from the Skagit Basin.

2. 3 Hatchery Smolt Release Impacts on Natural-Origin Steelhead Populations

A statistical analysis of steelhead population trends in the Skagit River was completed to assess the impacts of the hatchery steelhead smolt outplant programs conducted over the past 30 years on the abundance and productivity of natural-origin steelhead within the Skagit watershed and Puget Sound. Environmental factors that have been identified as possible causes for the decline in Puget Sound steelhead populations include habitat degradation and loss, increasing hydrological variability in rivers and streams, shifting ocean conditions, harvest (albeit at low levels in the Puget Sound), increased predation, and hatchery programs. The objective of this project was to identify the potential long-term impacts of latter factor, hatchery programs, on steelhead returns in the Skagit River. This was accomplished by statistically identifying potential sources of population variability from other environmental factors to wild Skagit steelhead returns, including ocean conditions and hydrological variability, and then controlling for these factors to identify and quantify the impact of hatchery smolt release programs on Skagit steelhead.

3.0 Natural-Origin and Hatchery Smolt Freshwater Outmigration Timing Characteristics

3. 1 Introduction

The timing and location of the annual release of Marblemount Hatchery smolts is controlled by WDFW hatchery staff each year. The basic premise of release timing has been to mimic the peak outmigration timing of their natural-origin counterparts. Over the past 20-years multiple release locations have been utilized. Since release year 2008 only two sites have been used; Marblemount hatchery and at the mouth of the Baker River near the PSE trap site. The vast majority of the smolts are released from the Marblemount Hatchery. The effects of the release size and timing on natural-origin smolts are unknown. The planned overlap in time and space of natural-origin and hatchery smolts during freshwater outmigration is an opportunity for competitive interactions to occur for habitat and food resources during the freshwater, estuarine and early marine stages of emigration. Other possible effects might take place as a result of predator attraction and hatchery smolt residualization. The resulting data will improve our understanding of spatial overlap between hatchery/natural-origin smolt as well as the outmigration period duration for each group.

3. 2 Methods

Possible overlap in freshwater outmigration timing between natural-origin and hatchery smolts was examined using WDFW smolt trap data from outmigration years 2007 and 2009 (Kinsel, 2008 and 2010). These years were chosen to represent a relatively small (2009) and large (2007) hatchery smolt release group. The smolt traps are located in the lower Skagit River mainstem near Mount Vernon at river mile (R.M.) 17 (Figure 3). Traps were operated from January through July in both years. All captured fish were enumerated by species and examined for external marks. The data presented is the daily raw (unexpanded) steelhead smolt catch data. Hatchery steelhead smolts are distinguished from natural-origin by the absence of the adipose fin.

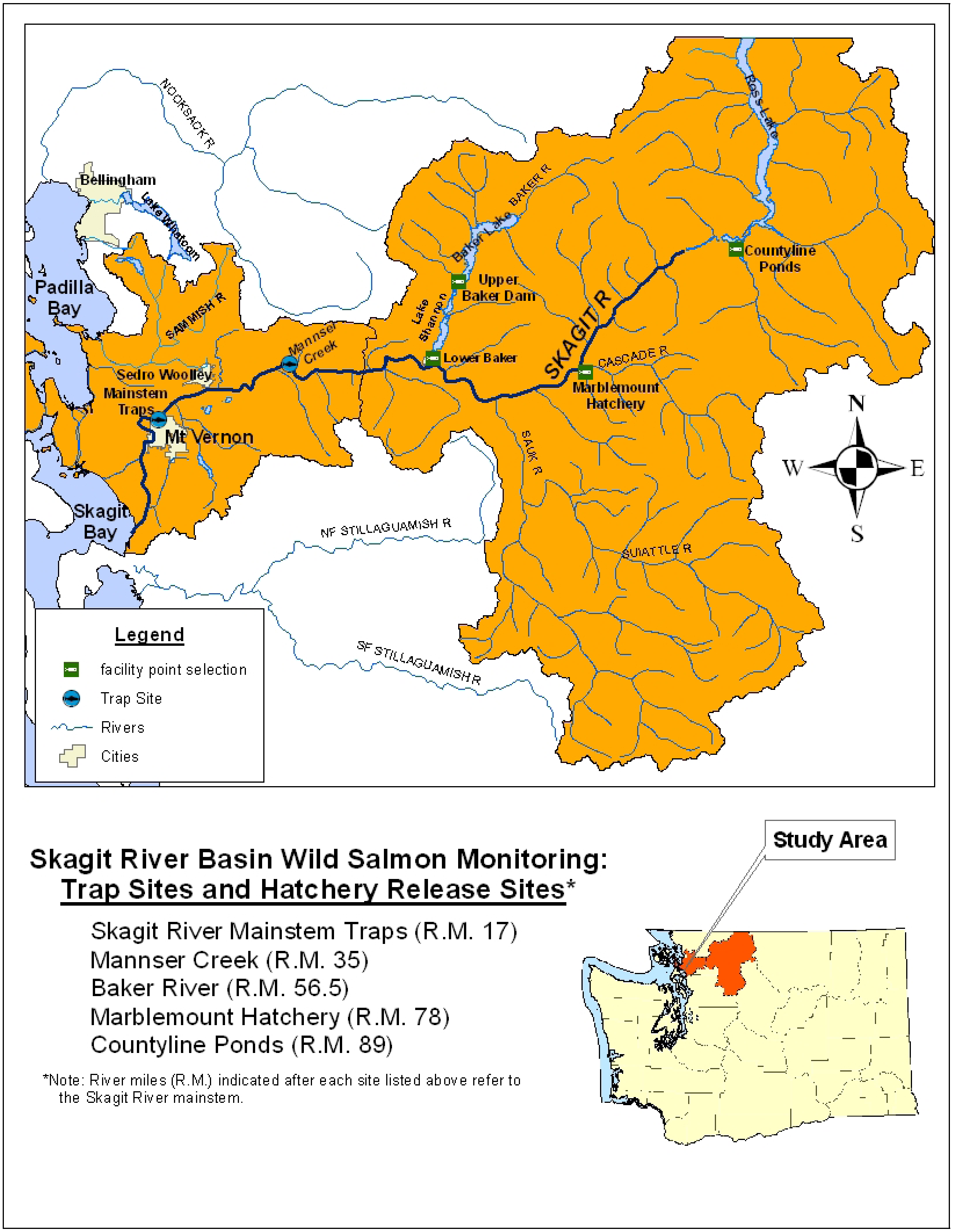


Figure 3. Map of tributary and mainstem trap sites and hatchery release sites in the Skagit River watershed. WDFW's Skagit River mainstem smolt trap is located near Mount Vernon at River Mile 17 (Courtesy of WDFW).

3. 3 Results

A total of 511,600 hatchery smolts were released between May 1-10 in 2007 and 174,000 were released at a slightly later between May 11-22 in 2009. The peak densities of hatchery and natural-origin steelhead captured at WDFW's Mount Vernon trap occurred very closely together during the spring outmigration periods of 2007 and 2009 (Figures 4, 5). The week of May 17 represented the peak week for both hatchery and wild origin smolts in 2007 while the 2009 peak for natural-origin was May 28 and a week later on June 4 for hatchery smolts. During both outmigration years the hatchery smolt outmigration period spanned a four week period while the natural-origin outmigration occurring over a much longer period of time beginning in January and persisting through early July in 2009. The data show an overlap in hatchery and natural-origin smolt riverine outmigration timing during the outmigration peak of the natural-origin smolts. The 2009 hatchery release was made approximately 10-days later than the 2007 release resulting in a slightly delayed arrival at the trap site and with the peak occurring nearly a week later than that of the natural-origin smolts. The magnitude of the hatchery smolt daily catches varied greatly between outmigration years paralleling the 66% reduction in smolts released in 2009. During the same two outmigration years the magnitude of the natural-origin catches varied little from each other.

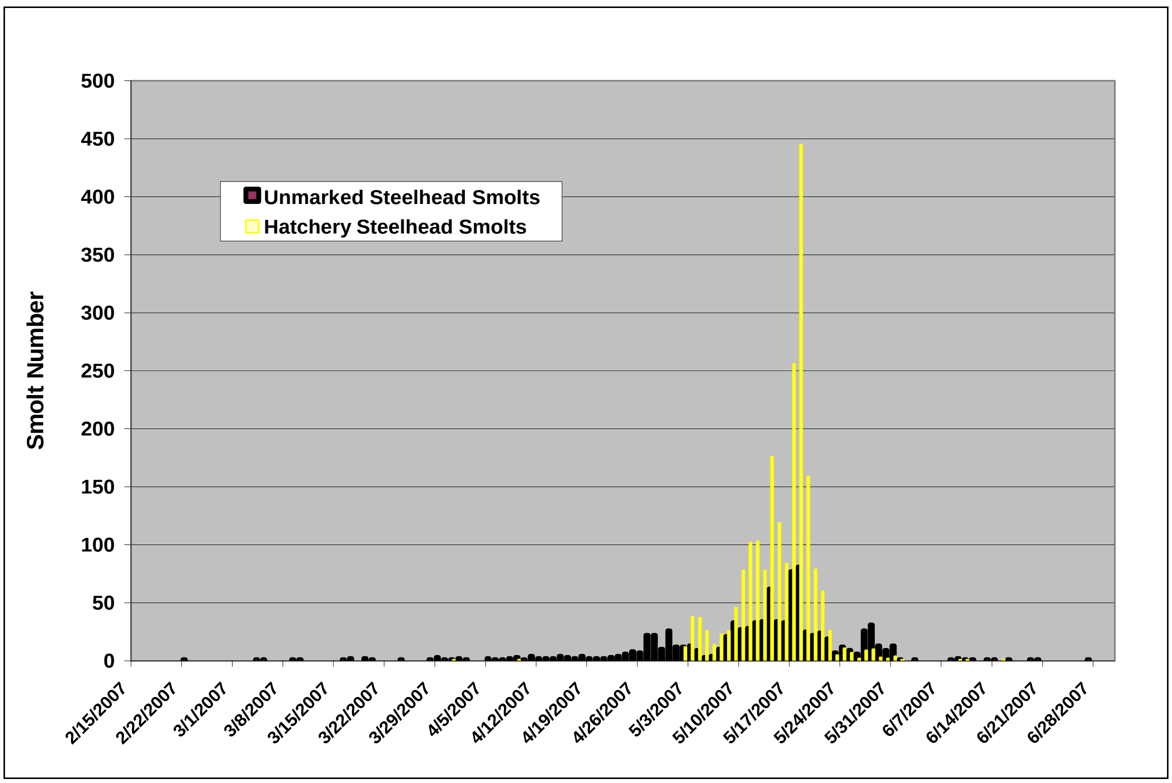


Figure 4. Hatchery and natural-origin steelhead daily smolt catch at the Mount Vernon smolt trap during the 2007 outmigration period.

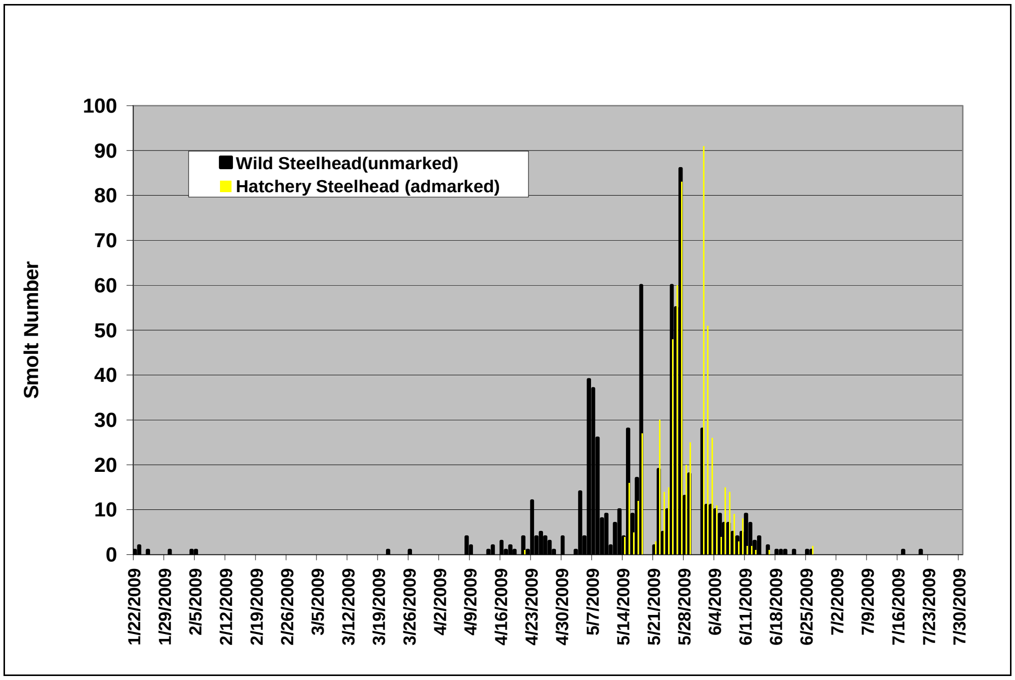


Figure 5. Hatchery and natural-origin steelhead daily smolt catch at the Mount Vernon smolt trap during the 2009 outmigration period.

3. 4 Discussion/Conclusions

Matching the peak of the natural-origin outmigration each spring with an early May hatchery release timing is meeting the current WDFW management objective as measured at the Mount Vernon smolt trap. This outcome creates an overlap in freshwater outmigration timing and habitat space utilization between natural and hatchery-origin smolts. During the 2-4 week freshwater outmigration period exhibited by hatchery smolts space and food resources are shared with their natural-origin counterparts. Although the effect of this release pattern on natural-origin smolts remains unclear it undoubtedly creates an opportunity for competitive interactions to occur for habitat and food during the freshwater, estuarine and early marine stages of emigration. The density of hatchery smolts present at the trap varies annually as was reflected in the daily smolt count levels. The 2007 hatchery release was nearly three times larger than 2009. This was exhibited by a comparison of the highest recorded daily trap counts between years. In 2009 there were no daily counts exceeding 100 smolts while in 2007 there were seven daily counts exceeding this level including a peak count of 440 smolts. In comparison, the natural-origin smolt peak daily counts were comparable between years suggesting that their densities during the peak outmigration period and perhaps smolt production levels were perhaps equivalent. This dataset demonstrates that variation in hatchery release size is detectable with the current trapping system. Assuming trap catch efficiencies are roughly similar for hatchery and natural-origin smolts the data from these two years allows for a rough comparison of hatchery and natural-origin smolt population magnitude occupying habitat and competing for food during the freshwater outmigration stage. In 2007 there appeared to be a much higher ratio of hatchery:natural smolts present compared to 2009 when the ratio was more balanced. If competition for food resources and space is occurring during the freshwater migration stage it is most extreme around the peak of the natural-origin outmigration period and in some years like 2007 the hatchery smolt densities are far greater than that of the corresponding natural smolts. During years of high hatchery smolt production, such as 2007, the level of competitive interaction with natural-origin smolt is elevated due to the higher densities of hatchery smolts relative to those of their natural-origin counterparts.

A better understanding of natural-origin smolt production levels is needed to help inform fish managers attempting to find the best balance in hatchery and wild production as described in HSRG (2004). The HSRG Principles and Recommendations document identified several important considerations in determining the proper size of a segregated hatchery program such as being used at the Marblemount Hatchery. In general, the number of fish released should be the smallest number necessary to meet the management goal of the program while avoiding the potential for ecological interactions with natural populations. Hatchery programs sized incorrectly present ecological risks such as those described here. For example, large hatchery releases may interact through competition and predation with natural stocks and other ecological processes in a detrimental way. These “extra” fish may also impact the survival of other populations once they enter the marine areas.

From an ecological standpoint the hatchery production level should be sized such that it is of a smaller magnitude than that produced by their natural-origin counterparts. For some years, such as 2007, the hatchery release numbers appear to greatly exceed that produced by natural-origin population. From an ecological standpoint the possible negative effects of this likely extend through the freshwater and marine migrant lifestages.

Freshwater residualization of both hatchery and natural-origin steelhead juveniles is well documented by other researchers (Quinn 2004). Residual hatchery smolts create another level of ecological interaction with natural-origin juveniles including competition for food and space as well as possible predatory interaction during extended periods of freshwater rearing. The presence and extent of hatchery smolt residualization has not been studied on the Skagit however they have been detected outside the typical outmigration period by other researchers (Lowery 2013).

Large releases of hatchery smolts in compressed time frames attract predators and create elevated levels of predation on both hatchery and natural-origin smolts present in the same migratory pathway (Lowery 2009). Lowery found that adult bull trout annually consume considerable numbers of Mykiss juveniles in the Skagit River during the outmigration period.

If natural-origin steelhead recovery is a high priority to fish managers, a reduction in the size of the Marblemount hatchery program may be warranted to reduce or eliminate ecological interactions with natural-origin juveniles during the freshwater and marine lifestages.

Another management option that could be used to further reduce ecological interactions with natural-origin smolt would be to alter the release timing of hatchery migrants. A delayed release would effectively minimize the overlap in time and space during the freshwater and marine lifestage. Negative ecological interactions between these two groups could largely be eliminated by creating a separation in outmigration timing. A delayed release time would largely eliminate any existing competition for space and food resources. This action should create the potential for improved growth and survival in both freshwater and early marine environments by natural-origin smolts.

4.0 Natural-Origin and Hatchery Smolt Freshwater Outmigration and Early Marine Residency Duration, Pathways and Survival

4. 1 Introduction

Our studies make an attempt to better understand behavior patterns of natural-origin and hatchery smolts during freshwater outmigration and early marine residency. The duration, pathways and survival determined for these sub-periods were used to measure similarities and differences in behavior between hatchery and natural-origin outmigrants. The extent of interactions or overlap in time and space between these two groups will help us understand potential impacts to the natural-origin outmigrant population caused by hatchery produced outmigrants. The presence, abundance and release timing of hatchery smolts could potentially affect growth and survival of natural-origin outmigrants due to competition for food resources or space. It is also unclear how known predators react to the presence/absence or magnitude of hatchery-origin migrants.

We used biotelemetry technology to study the riverine, estuarine and marine migratory behavior and survival of natural-origin and hatchery-reared steelhead smolts descending the Skagit River during the 2008 and 2009 outmigration periods. More specifically, differences in travel time and migratory behavior of these two groups were examined. All hatchery smolts originated from the Marblemount hatchery facility and were tagged and released directly from the hatchery or at up to three other sites within the basin as discussed in the methods section. Natural-origin steelhead smolts were captured and tagged at the smolt trap near Mount Vernon in the lower Skagit River. Attempts to capture and tag natural-origin smolts in the upper watershed (upper Skagit) were unsuccessful, and this limited migrational comparisons between hatchery and natural-origin smolts in freshwater riverine corridors.

4. 2 Methods

4.2.1 Hatchery Smolts

A total of 50 hatchery smolts of Chamber's Creek origin were acoustically tagged in 2008 at the Marblemount hatchery and released with release groups at four different locations (Table 1). Smolt size averaged 182mm at approximately 6 smolt/pound. Vemco V7-2L tags were used for both natural and hatchery smolts during the 2008 outmigration period. All tags were individually-coded and transmitted pulse trains at 69 KHz frequency, 15–45 second random delay (VEMCO Ltd, Halifax, Nova Scotia Canada). The rated battery life of the transmitters as specified by the manufacturer was 52 days. Based on actual tag detection data, battery life on many fish exceeded 30 days.

Tagged fish were held in isolation by tag group for a 3-4 days to determine any tag shedding or post-tag mortality. After the short isolation period each tag group was placed in a rearing pond with their respective release group to acclimate with each other for a few days prior to release date.

Table 1. Hatchery smolt tagging details for 2008 outmigration year.

Hatchery Rearing Site	Release Location	Release Date(s)	River Mile	Release Group Number	Number Tagged
Pond 23	Marblemount Hatchery	May 12	78.1	185,000	20
Pond 21	Barnaby Slough	May 12	70.2	20,000	10
Pond 23	Baker River	May 12-13	56.4	30,000	10
Pond 23	Sauk River	May 13	78.5*	10	10

*River mileage includes 66.5 mile of mainstem Skagit River

A similar experiment was repeated in 2009 using slightly larger and more powerful tags to improve tag detection rates. On April 23, 2009 fifty-five Marblemount hatchery smolts (Chambers Creek origin) from the 2007-8 brood were surgically implanted with larger V9-2H acoustic transmitters. Smolt size ranged from 170-200 mm (6-9 fish/pound [50-75 g/fish]). Tags were individually-coded and transmitted pulse trains at 69 KHz frequency, 15–45 second random delay (VEMCO Ltd, Halifax, Nova Scotia Canada). The rated battery life of the transmitters, as specified by the manufacturer, varied from 45 days for short interval tags and 77 days for tags with long interval. Based on actual tag detection data, battery life on many fish exceeded 36 days. The longest detection period of any fish was 50 days, suggesting the battery life extended into the middle of the rated battery life range. The release groups consisted of a release directly from the hatchery, another near the mouth of the Baker River, and a control group, which consisted of 5 tagged smolts held at the hatchery for an additional 14-day period to evaluate delayed tag mortality and battery life before being released directly from the hatchery (Table 2). All of the control fish survived and tag function was confirmed prior to final release. The control group confirmed that these fish survived for at least a 4-week period in the hatchery and that the tags continued to function properly.

Table 2. Hatchery smolt tagging details for 2009 outmigration year.

Tag Group Based On Release Location	River Mile	Number Tagged	Release Group Size	Release Date
Marblemount Hatchery	78.1	25	125,000	May 12, 2009
Baker River Mouth	56.4	25	28,000	May 11, 2009
Marblemount Hatchery Control Group (Delayed Tag Mortality)	78.1	5	21,000	May 22, 2009

For both release years, tags were programmed to remain on for a 24-hour period upon activation (surgery day) followed by 14-days of deactivation and finally reactivated just prior to release date and remained functional until battery life was extinguished. After a 14-day isolation period to confirm tag reactivation and assess direct tagging-related mortality, tag extrusions, and monitor for signs of impaired swimming behavior the three tag groups were integrated into their respective release site group 3-days prior to volitional release (Table 1, 2).

It should be noted that an estimated 90% of the smolts released from the hatchery do so within a few hours (Personal communication Steve Stout [Marblemount Hatchery Manager]).

Acoustic tags were surgically implanted in these fish using established protocols (Adams 1998). The same highly experienced surgeon performed all surgeries. Fish were anaesthetized and placed ventral side up on a foam surgery board. The gills were continuously irrigated with a gentle flow of water containing a maintenance dose of anesthetic throughout the procedure. Tags were inserted through a mid-ventral incision and closed with two or three sutures.

4.2.2 Natural-Origin Smolts

During 2008 and 2009, 43 and 25 natural-origin smolts were captured, acoustically tagged and released at the Mount Vernon smolt trap (see Figure 3). In 2008 the fish were tagged and released between May 28 and June 5 and from May 12-18 in 2009.

The same surgical protocols described for hatchery smolt tagging were used for natural-origin riverine migrants. All natural-origin smolts were implanted with V7-2L acoustic transmitters. The rated battery life of the transmitters as specified by the manufacturer is 52 days. Based on actual tag detection data, battery life on many fish exceeded 30 days.

Steelhead smolts were collected at the trap and held on site until adequate numbers had been accumulated for surgeries. Tagged smolts were generally held for 2-3 hrs after surgery to assess direct tagging-related mortality, tag extrusions, and monitor for signs of impaired swimming behavior. These were rarely observed. All fish were released directly from the smolt trap location (RM 17) after the holding period. Capture and tagging occurred during the months of May and early June in 2008 and May only in 2009. The size metrics for tagged smolts varied between years (Table 3). The smolts from 2008 were slightly smaller in both length and weight than those used in 2009.

Table 3. Natural-origin steelhead smolt size metrics.

Outmigration Year	Average Length (mm)	Length Range (mm)	Average Weight (grams)	Weight Range (grams)
2008	165.2	147 - 200	48.85	34.2 - 96.5
2009	184.1	150 - 240	68.8	118.5 - 39.5

4.2.3 Acoustic Receiver Array

Acoustic receivers (69 kHz VEMCO VR-2 or VR2W models) were used to track all tagged downstream migrants in both freshwater and marine areas. Receivers were arrayed throughout the Skagit River watershed, near-shore marine areas, and northern Puget Sound as shown in Figures 6 and 7. In marine areas, receivers were placed individually in key migrational zones or arranged in lines across the Juan de Fuca Strait (JDF) and Deception Pass (Figure 6). The lines were constructed to provide sufficient overlap in detection radii of receivers so that most tagged adults and smolts would be expected to transmit at least a few signals while crossing each line.

The freshwater portion of the array spans the Skagit River delta and extends into the upper watershed sub-basins (Figure 7). Receiver deployment locations were selected such that subbasin and in some cases tributary pathways could be identified. The freshwater receiver array consisted of 27 receivers within three sub-basins and two tidal estuarine zones on a year round basis.

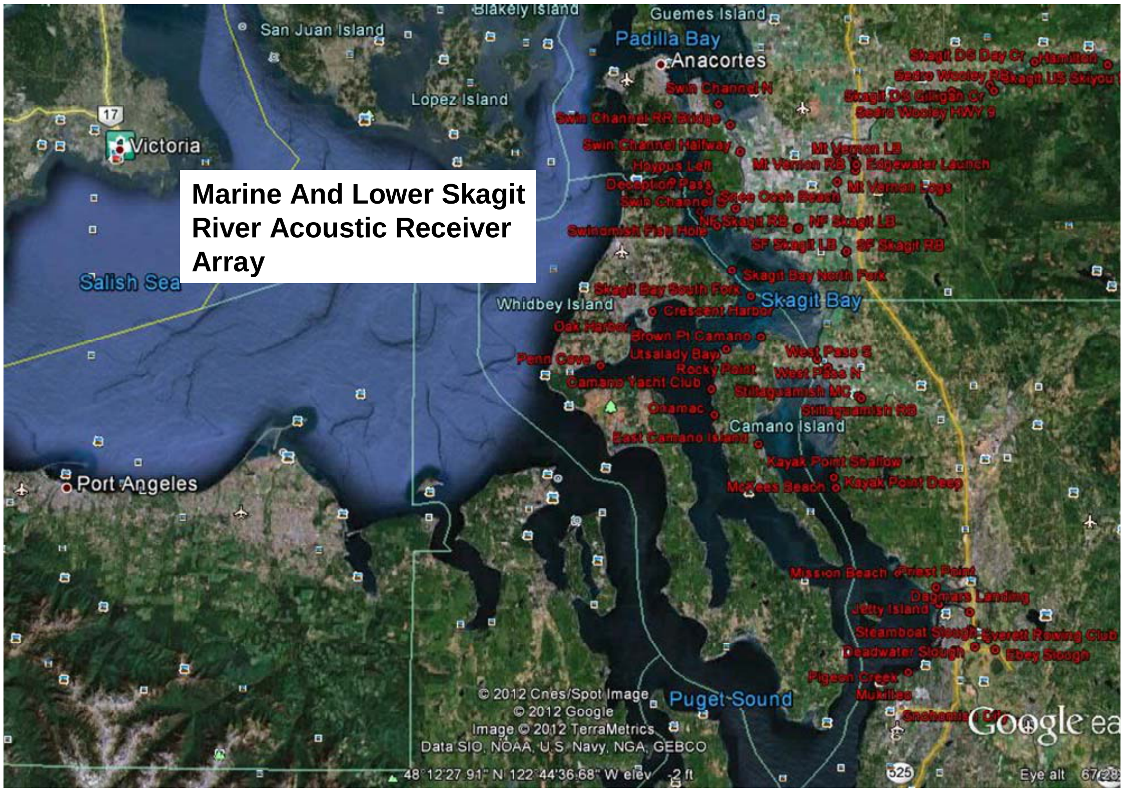


Figure 6. Marine and lower Skagit River acoustic receiver array.

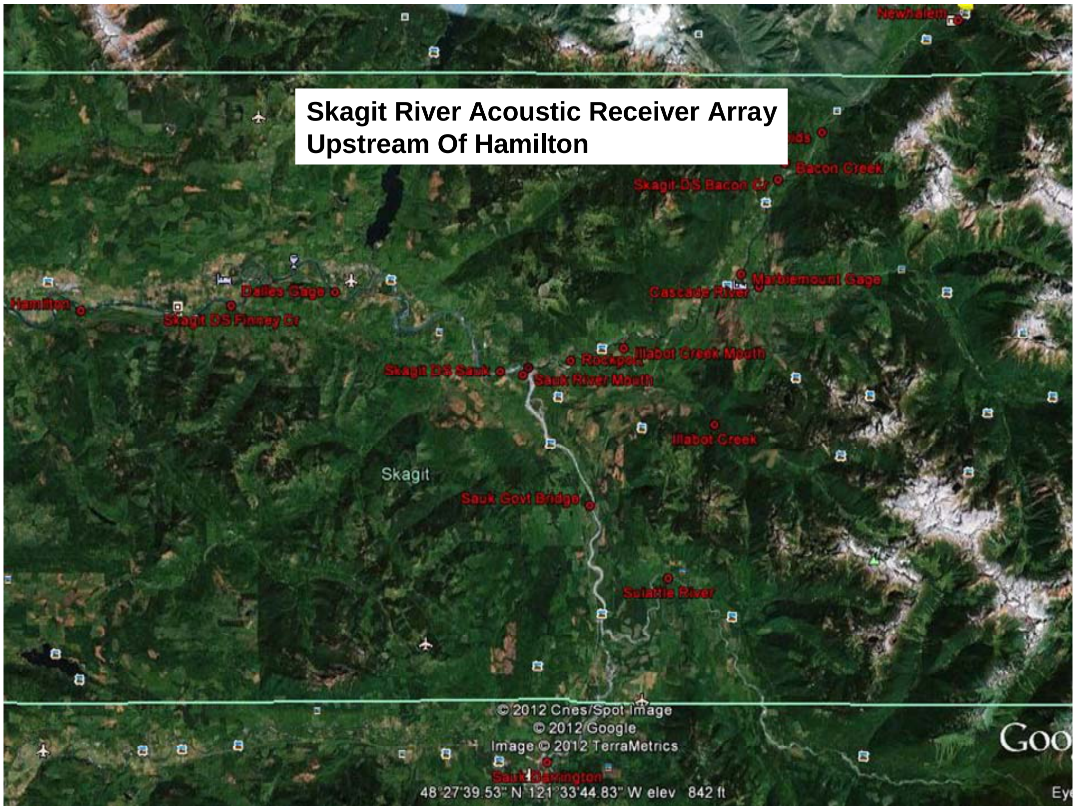


Figure 7. Skagit River acoustic receiver array upstream of Hamilton.

Smolt detection efficiencies varied greatly by tag type, year, and detection environment (Table 4). The marine receiver detection rates varied from 16% to 71% for smolt steelhead. The V7 tags were largely ineffective in the freshwater portion of the array producing almost no detections in 2008 until fish entered tidal areas (Table 4). Detection efficiencies exceeding 70% were observed in 2009 after using larger V9 tags. The V7s used for natural-origin smolt resulted in far lower detection rates than the larger V9 tags used exclusively with hatchery smolts in 2009.

Table 4. Acoustic tag detection rates by year, tag type and water type.

Tagging Group	Tag Type	Freshwater Detection Efficiency	Marine Detection Efficiency
2008 Hatchery Smolts	V7	2%	16%
2008 Natural-origin Smolts	V7	0%	54%
2009 Hatchery Smolts	V9	76%	71%
2009 Natural-origin Smolts	V7	36%	40%

4.2.4 Data Analysis

We compiled a database of detections from the acoustic array consisting of tag identification, detection date/time and location. False positives, due to tag code collisions or other noise sources, were removed from the database. Detections of fish were also excluded as false if they were detected only once on a line within 60 minutes, had one or more tags heard on the same receiver around the time of the suspect detection, and did not have supporting detections from other time periods or lines. Supporting detections are defined as a temporal sequence of detections from the release date along the migration path. After suspect detections were eliminated the filtered data were used to establish migration or movement pathways and estimate travel times and survival of tagged smolt during downstream freshwater and marine migration.

Mean travel times of release groups or individual fish were calculated for freshwater (from release site to detection near the river mouth) and marine (from river mouth to marine locations and line at Strait of Juan de Fuca) portions of the outmigration. These were measured as the time from release until the first detection of a tag at individual river receiver locations, river mouth or marine station, and were averaged across fish within each population group. Travel times were measured as the difference between successive receivers or lines in the cumulative travel times from release until the first detection of a tag at a receiver or on a line. In addition, because of different release locations, only hatchery smolts detected at Mount Vernon receiver locations (near smolt trap location) were used to compare 2009 hatchery with natural-origin smolt marine travel times.

4. 3 Results

4.3.1 Freshwater Outmigration

Fifty Marblemount hatchery smolts, of Chambers Creek origin, were tagged in 2008 and released with their release group at four locations. The detection performance of the tags used 2008 was so poor that no useful data was recovered from the freshwater portion of the array. During the 2009 outmigration period another 55 hatchery smolts were tagged with a more effective tag and released from two locations; Marblemount hatchery and the mouth of the Baker River. Overall 76% of those tagged were detected either in-river or near the mouth of the Skagit River (Table 5). Seventy- three percent of these smolts succeeded in reaching marine waters. Because the detection efficiency of the receiver array is unknown, the minimum freshwater survival rate of this group is 76%. Regardless of release location and time of release smolts detected leaving the river spent between 11-30 days before entering Skagit Bay. The average outmigration period for the three release groups averaged between 19.4 - 22 days. Of those smolts failing to be detected in marine waters, two were last detected in the Skagit River but were never detected in marine receiver array and another 13 tagged smolts were never detected by either the freshwater or marine portion of the receiver array. There are several possible freshwater outcome alternatives for these 15 smolts that include; evasion of the freshwater array, not expressing anadromy (in-river residuals), fell victim to predators, or died before travelling far enough to pass a receiver location.

The plan to acoustically track smolts of natural-origin was unsuccessful due to difficulty in capturing smolts in up river locations. Similar outmigration acoustic data during freshwater outmigration could not be generated for natural-origin smolts in the upper Skagit and Sauk rivers during the spring of 2008 and 2009. For this reason travel time, pathway and survival comparisons between natural and hatchery-origin smolt in the freshwater corridor were not possible.

Table 5 - Freshwater residency time during outmigration of 2009 hatchery smolts.

Release Location	Number Tagged	Number Detected	% Detected	Average Days to Bay	Range
Marblemount	25	20	80%	19.4	11-30
Baker	25	19	76%	20	12-28
Control Group	5	3	60%	22	10-31
Total Hatchery	55	42	76.4%	20.4	11-30
Wild @ Trap	25	14	56.0%	-	-

4.3.2 Early Marine Residency

Hatchery-origin smolts tagged in 2008 were not detected effectively in the freshwater portion of the receiver array so it was not possible to determine marine entry date and time for individual fish. Because of this, the early marine residency data shown in Table 6 below for 2008 hatchery smolts express elapsed time from point of release to each marine detection location. The remainder of the data in Table 6 reflects true marine travel times from Mount Vernon to each of the four marine detection locations shown. For this reason it appears that the 2008 hatchery smolts took considerably longer than the 2009 hatchery smolts to arrive at each location. The 2008 hatchery smolts required 16-29 days to travel from their associated point of release to the Strait of Juan de Fuca (SJF) receiver line. The 2008 natural-origin smolts released at the Mount Vernon smolt trap spent on average 18 days to travel to the SJF line. As might be expected, smolts of both hatchery and natural-origin taking the shorter Deception Pass route to SJF spent considerably less time reaching SJF than those choosing the longer southerly route around the south end of Whidbey Island.

Unlike the previous year, the data from natural and hatchery-origin smolts tagged in 2009 did allow for direct comparison of travel time to each marine detection location. With the exception of the Admiralty Inlet detection point, hatchery-origin smolts required less time than their natural-origin equivalent to reach both Deception Pass and SJF. Natural-origin smolts averaged 18.3 days to travel to SJF while hatchery-origin smolts spent from 5.3 to 13.5 days depending on release location group (Table 6), suggesting that hatchery-origin smolts likely spent less time reaching SJF overall in 2009.

When comparing the marine travel times between years for natural-origin smolt groups the results show that the time needed to reach each marine detection point was almost identical. Overall, smolts of hatchery-origin appear to spend less time reaching both Deception Pass and SJF than their natural-origin counterparts.

Table 6. Marine residence time expressed in average number of days for natural and hatchery-origin smolt leaving the Skagit River in 2008 and 2009 outmigration periods at four marine locations.

Year/Release Location	Deception Pass	Possession Bar	Admiralty Inlet	Strait of Juan de Fuca
Wild 2008/Mount Vernon (b)	5.8	9.3	14.2	17.8
Hatchery 2008/Marblemount Hatchery(a)	15.5	n/d	18.0	27.0
Hatchery 2008/Barnaby Ponds(a)	12.5	19.0	23.2	22.0
Hatchery 2008/Baker River Mouth(a)	n/d	n/d	n/d	16.0
Hatchery 2008/Sauk River(a)	15.0	n/d	n/d	28.9
Wild 2009/Mount Vernon	5.4	10.5	14.4	18.3
Hatchery 2009/Marblemount Hatchery(b)	2.1	n/d	n/d	5.3
Hatchery 2009/Baker River Mouth(b)	3.1	n/d	18.3	13.5
Hatchery 2009/Hatchery Control(b)	2.3	n/d	n/d	6.9
(a) Days from respective release sites				
(b) Days from Mount Vernon (adjusted)				

4.3.3 Tidal and Marine Migratory Pathways

From the mainstem of the Skagit River at Mount Vernon (RM 16) there are two outmigration routes through either the south or north forks to the marine zone of Skagit Bay. Once a smolt enters Skagit Bay there are two main routes to SJF from the Skagit River mouth. The northern route involves passing through Deception Pass at the north end of Whidbey Island. The alternate route involves traveling south around the southern tip of Whidbey Island. Of these two routes the southern route is the longest and most indirect.

Only limited data were obtained for natural-origin smolts because of the poor detection efficiency of the tags used throughout the study. In contrast, a much larger marine dataset was created for hatchery-origin smolts in 2009 because of the higher detection efficiency of the larger tags. This yielded far more information on marine migratory pathways for hatchery smolts. The 2009 dataset showed that nearly equal numbers of hatchery-origin smolts utilize each fork of the river to reach Skagit Bay (Table 7). Of the twenty-eight hatchery-origin smolts detected taking the southern or northern route to SJF 93% followed the northern route through Deception Pass. This result differs from the results derived from a more limited natural-origin dataset in that there was an even split between the northern and southern routes to SJF (Table 7). Unfortunately in-river detection efficiency was so poor for natural-origin smolts that only one was detected traveling down the south fork to Skagit Bay. There was insufficient data to determine whether natural-origin smolt have a lower river pathway preference.

Table 7. Migratory pathway tendencies of natural and hatchery-origin smolts during the early marine migration period based on acoustic tracking data from the Skagit during the 2009 outmigration year.

Smolt Group	Release Location	Sample Size	North Fork Skagit	South Fork Skagit	Deception Pass	South Whidbey	Strait of Juan de Fuca
Wild	Mount Vernon Trap	25	-	1	3	3	1
Hatchery	Marblemount Hatchery	25	5	5	12	-	9
Hatchery	Baker Mouth	25	8	7	12	2	6
Hatchery Control	Marblemount Hatchery	5	2	1	2	-	2

4.3.4 Marine Survival

The number of tagged fish arriving at each freshwater and marine detection site from Mount Vernon to SJF served as an unadjusted measure of survival. A more accurate measure of survival could be achieved by adjusting for detection efficiency at each site. Estimated detection efficiencies for the tags types used in this study have been developed by other researchers for the SJF and Admiralty receiver lines. There are no tag detection efficiency levels developed for the lower Skagit River and at Deception Pass line. The survival estimates contained in Table 8 are based on actual, unadjusted detections at each of the three locations shown; tidal affected area of the lower Skagit River, Admiralty line located on the western side of Whidbey Island and the SJF line. The survival levels shown are a minimum survival rate since they underestimate actual survival (some fish pass each site without being detected). This is especially the case for natural-origin smolts that were tagged with smaller more poorly detected tags. It should be noted that the Whidbey basin survival rates were higher than the corresponding freshwater survival rates for wild smolts and hatchery smolts released at the Marblemount hatchery. In these two cases the freshwater array detection efficiencies were less than observed in the Whidbey basin. In both cases the observed freshwater survival underestimated actual.

The freshwater survival of hatchery smolt, from point of release to Skagit Bay, varied by release site and ranged from 40-60% (Table 8). Marine survival to SJF also varied by release group and site ranging from 24-40%. The Marblemount release group lost an additional 4% while those released at Baker River mouth dropped an additional 36%.

Survival of natural-origin smolts was considerably lower than what was reported here for hatchery-origin smolts. This is largely explained by the poor detection efficiency of the tags used for these fish in contrast to the more effective tags used with hatchery smolts. Only 4% of the natural-origin smolts were detected at the SJF line. When detection efficiency is factored into these findings the survival levels are consistent with results from other researchers evaluating steelhead smolt survival to SJF (Moore, 2010).

Table 8. Minimum survival rates of natural and hatchery-origin smolts during the 2009 outmigration year in freshwater, Whidbey basin and to the western extent of the Strait of Juan de Fuca (not adjusted for detection efficiency)

Smolt Group	Release Location	Sample Size	Freshwater	Whidbey Basin	Straits of Juan de Fuca
Wild	Mount Vernon Trap	25	4.0%	12.0%	4.0%
Hatchery	Marblemount Hatchery	25	40.0%	48.0%	36.0%
Hatchery	Baker Mouth	25	60.0%	56.0%	24.0%
Hatchery Control	Marblemount Hatchery	5	60.0%	40.0%	40.0%

4. 4 Discussion/Conclusions

By studying the behavior patterns of migrant natural and hatchery steelhead during the freshwater outmigration and early marine residency periods we felt it would be possible to determine the extent of possible interactions or the overlap in time and space between these two groups. In addition, any measure of hatchery smolt survival during this lifestage would further help define the magnitude of possible interaction. Higher survival equating to more potential ecological interactions with those of natural-origin assuming both groups occupy the same space.

For outmigration year 2009 there were two separate measures of hatchery smolt outmigration timing and duration. Individual fish were monitored with acoustic tags and the travel time of each hatchery release group could also be coarsely measured by using their arrival time at the Mount Vernon trap. During the freshwater outmigration sub-period hatchery travel time averaged approximately 20-days to reach Skagit bay. Some arrived within 10 days while other took up to 30 days. For the same 2009 migrant group, based on hatchery release dates, first migrants arrived at the trap within 5 days of release. The bulk of the hatchery migrants passed the trap within 18-24 days of release and the last captures occurred more than 30 days post release. The freshwater duration results from individually tracked and smolt trap captured (untagged hatchery smolts captured at the trap) migrants were comparable providing confirmation that hatchery migrants spend from 10-30 days traveling from the hatchery to Skagit bay. Unfortunately similar freshwater duration data is not yet available for natural-origin migrants. However, smolt trap data shows that natural-origin migrants are also present in the freshwater migration corridor while hatchery release groups are outmigrating. Skagit smolt trapping data demonstrates that hatchery release timing assures that outmigration occurs during the peak of natural-origin migration as described in Section 2.4.

The extent of interactions between hatchery and natural-origin migrants in the freshwater corridor is also dependent on number released and their survival. In 2009, the acoustic data revealed that at least 73% of the hatchery smolts tagged get through to the marine environment. Given the most current levels of smolt release, at least 130,000 – 365,000 hatchery migrants are traveling down the mainstem corridor when natural-origin smolts are at their peak densities. Any ecological interactions between these two groups are occurring when both are at their highest densities. These interactions could potentially affect growth and survival of natural-origin outmigrants due to competition for food or space. In addition, it is also unclear how known predators, such as bull trout, react to the presence/absence or magnitude or release timing of hatchery-origin migrants.

As with the freshwater stage, the early marine residency times were used to establish the extent and duration of interactive overlap potential. When comparing the marine travel times between years for natural-origin smolts the results show that the time needed to reach each marine detection location was strongly consistent for both years (Table 6). Natural-origin migrants spent approximately 18 days traveling through the Whidbey basin outward to the detection line at the westerly end of the Strait of Juan de Fuca. Because hatchery freshwater detections were poor in 2009 it was not possible to determine a freshwater departure date. Without departure dates for individual fish a true marine residency period comparison between natural and hatchery-origin migrants could not be made. The only valid comparison possible was for the amount of time spent between Deception and JDF line. In 2008 hatchery migrants spent 2-10 days arriving at JDF line while in 2009 the time frame increased to 9-14 days. The natural-origin fish varied little between years averaging 12 days in 2008 and 13 days the following year. The data seems to suggest that depending on the year hatchery-origin smolts can spend less time reaching SJF than their natural-origin counterparts but generally spend equivalent amounts of time inside the Whidbey basin to JDF marine zone. From an ecological interaction standpoint it again appears that both groups occupy the same marine habitat although the dimensions of the marine zone are much larger than within the freshwater corridor. In general, it appears that there is an overlap in space and time which has the potential to effect growth and survival of natural production migrants.

The migratory pathway chosen by each group can affect the potential for and magnitude of interactions and shared resources. During both years the hatchery migrants clearly showed a preferred pathway as compared to the natural-origin migrants. Slightly more than 93% of the hatchery migrants took the shorter route through Deception Pass as opposed to the longer route around the south end of Whidbey Island before traveling north toward the SDJ line. Although the data was derived from a much smaller dataset, equal numbers of the natural-origin migrants traveled each of the two pathways.

It is possible that the slightly longer observed residency period of natural-origin migrants may be due to the higher proportion of the population traveling the longer route around the south end of Whidbey Island. Since the preponderance of hatchery-origin migrants chose a different migratory route than their hatchery counterparts during the early marine residency stage, this behavior would reduce some of the ecological overlap occurring in marine areas.

The acoustic data indicates that more than 50% of the hatchery migrants are surviving inside the Whidbey basin and approximately 30% are reaching JDF before reaching the Pacific Ocean. As was the case in the freshwater migrant corridor, there are large numbers of hatchery smolts surviving in the marine residency zone to occupy the same marine habitat as the natural-origin population each year and to compete for the same food resources.

Nearly 24% of the hatchery smolts tagged in 2009 were never detected. The outcome alternatives of these hatchery migrants include; undetected in freshwater and entering marine waters, became stream resident, were preyed upon, or died. Other researchers have documented that some hatchery produced steelhead can and do take on an extended period of freshwater residency (Quinn, 2004). While the ultimate outcome of these unaccounted for hatchery juveniles remains unclear it is likely that some portion of the hatchery release become stream residents rather than exercise the anadromy lifehistory type. For non-migrants of this type the ecological implications are clear; direct competition with natural-origin juveniles for space and food resources as well as possible hybridization with natural-origin adults.

Our studies attempted to better understand behavior patterns of natural-origin and hatchery smolts during freshwater outmigration and early marine residency. The duration, pathways and survival during these periods were used to measure similarities and differences between hatchery and natural-origin outmigrants. The extent of interactions or overlap in time and space between these two groups in both the freshwater and marine migration zones demonstrate that there are clearly areas of potential impact on the natural-origin outmigrant population created by the presence and abundance of hatchery produced outmigrants.

5.0 Hatchery Steelhead Smolt Predation on Natural-Origin Steelhead Juveniles and Competition for Similar Diet Items

5. 1 Introduction

Natural-origin salmonids are subject to predation by other piscine predators as has been well documented by other researchers in the Pacific Northwest (HSRG 2004, Fresh 1997; Levin et al. 2001). Concern has also been expressed about hatchery reared steelhead preying upon wild juvenile salmonids, including natural-origin mykiss (HSRG, 2004). The impact of predation of this type may contribute to future difficulties in the recovery of threatened natural-origin steelhead in the Skagit River.

In the case of the Skagit watershed, predation by hatchery steelhead smolts, during the freshwater migration stage, on natural-origin mykiss juveniles was considered another possible form of ecological interaction between these two conspecifics. During the 2-3 week hatchery smolt outmigration period a variety of salmonid prey items are present in the migratory corridor consisting of 78 river miles of the Skagit River mainstem channel downstream of the Marblemount hatchery. Commonly occurring salmonid prey species present during the spring temporal period include Chinook, pink (every other year), coho and mykiss juveniles in both the migrant and pre-migrant stages. We studied the extent to which Marblemount hatchery steelhead smolts prey upon natural-origin Mykiss juveniles during their freshwater outmigration lifestage sub-period.

5.2 Methods

To determine whether hatchery steelhead smolts prey on natural-origin steelhead juveniles or compete for similar diet items the stomach content of Marblemount hatchery smolts were examined from fish captured at the smolt trap located in the lower Skagit River. The WDFW-operated mainstem smolt trap captures several hundred natural and hatchery-origin steelhead each year. WDFW staff collected hatchery smolts for stomach samples from their Mount Vernon smolt trap located at RM 15.6. All specimens were identified as hatchery-origin (ad-clipped) and of typical hatchery size averaging 180.6 in 2009 and 180.0 mm in 2010. Each fish was measured in the field to the nearest 1 mm fork length (FL). Fifty hatchery-origin steelhead smolts were retained each year during the 2009 and 2010 spring outmigration period; May 14-25 and May 6-25 respectively. For both outmigration years Marblemount hatchery smolts were released from the same two locations during early May; the Marblemount hatchery and at the Baker River trap, 62.4 and 40.9 river miles upstream of the WDFW smolt trap located near Mount Vernon.

Stomachs were evacuated to determine prey item content. Stomach contents were hand-sorted in the laboratory. Prey items from each smolt were identified to the species level and order for terrestrial organisms whenever digestion state permitted. Disarticulated or partly digested fish prey items that could not be identified were placed in an unidentified fish category. Diet items were pooled and analyzed by numeric and frequency of occurrence methods.

5.3 Results

Fifty Marblemount hatchery steelhead smolts were collected in each of two years at the Mount Vernon outmigrant trap after having travelled downstream from two different release locations upstream. The stomach content of these fish were used to determine whether hatchery smolts prey upon natural-origin steelhead juveniles or compete for similar food types during the freshwater outmigration period.

In both years hatchery smolts preyed on a variety of items falling into two major categories, juvenile fish and insects of both a terrestrial and aquatic nature. Numerically, pink salmon fry dominated the diet during the spring outmigration period of 2010. With the absence of pink fry during the 2009 outmigration period much higher numbers of other salmonids such as chinook, chum, and coho and insects were consumed (Figure 7). Notably there was an absence of juvenile mykiss consumed in either year. Fish and aquatic insects comprised 99.6% of all diet items when both years are combined with terrestrial insects contributing an insubstantial numerical amount each year and mykiss juvenile contributing nothing (Figure 8).

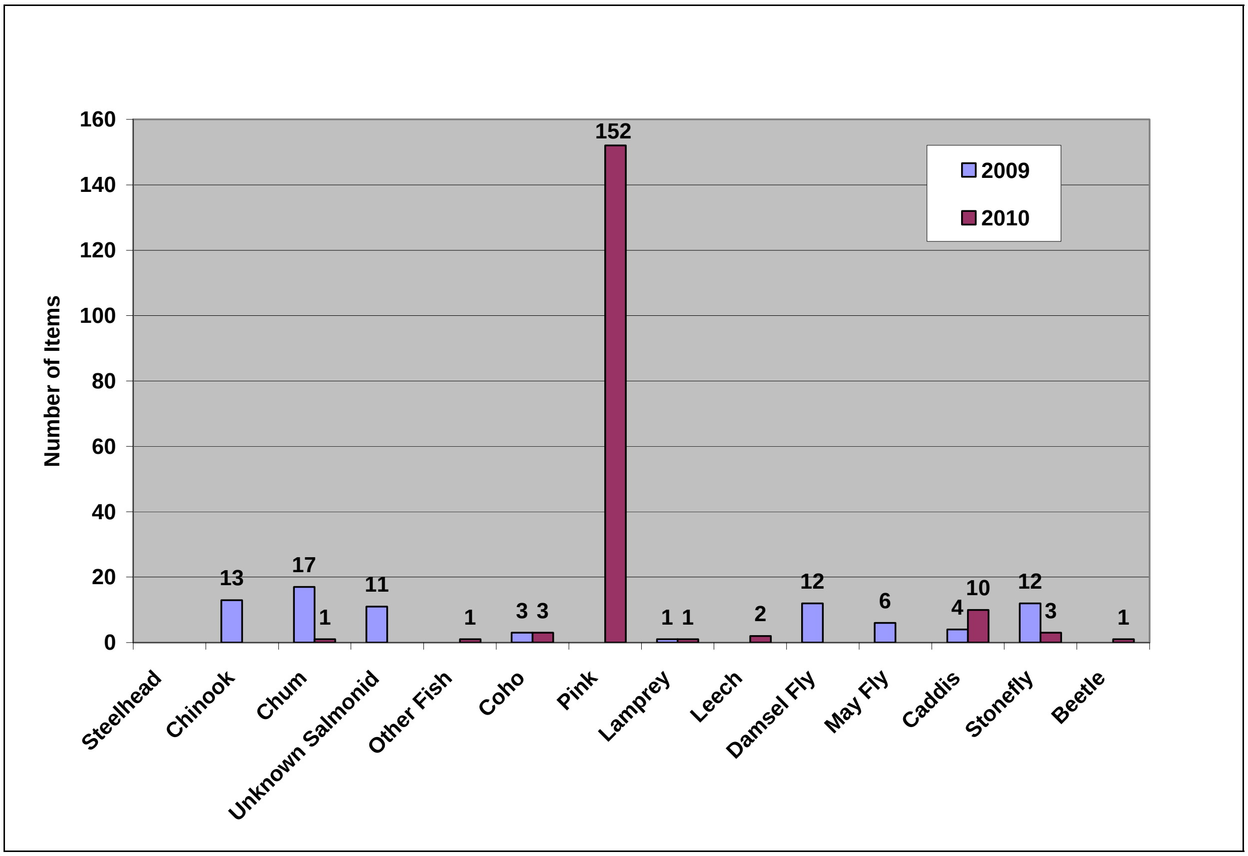


Figure 7. Marblemount hatchery smolt prey items during the 2009 and 2010 outmigration periods.

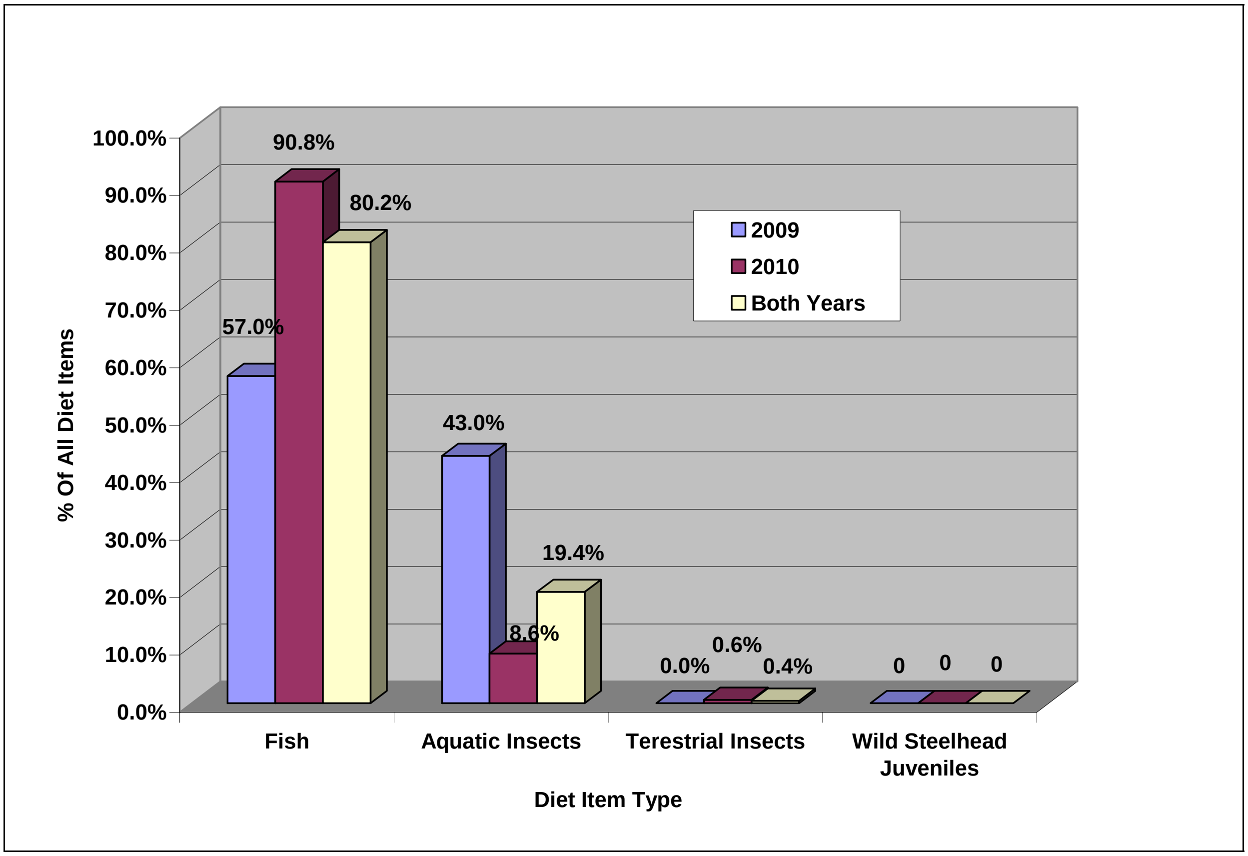


Figure 8. Marblemount hatchery smolt diet composition during the 2009 and 2010 outmigration periods.

5.4 Discussion/Conclusions

Predation on natural-origin mykiss juveniles by hatchery steelhead migrants is considered most likely to occur in the freshwater stage of migration because of exposure to large numbers of prey in a relatively small river corridor area. Several studies have reported that hatchery steelhead smolts prey upon wild salmonid juveniles. Cannamela (1993), Menchen (1981) and Beauchamp (1995) all reported that steelhead smolts, both wild and hatchery, were significant predators on naturally produced salmonids such as Chinook and sockeye fry.

Although there is evidence that predation of salmonid fry by migrating hatchery steelhead smolts may be common in streams, the estimation of risk to wild salmonid fry from predation in Washington streams is hindered by a lack of published data on the comparative feeding habits of hatchery and wild steelhead smolts.

It should be noted that the conclusions drawn from our dataset were derived from only a modest sample size from each of two consecutive outmigration years. Given this, it appears hatchery smolts, in only the first few weeks since hatchery liberation, are both indiscriminate and opportunistic predators judging from the variety of prey items encountered. Notably there was an absence of natural-origin juvenile mykiss consumed in either year. It appears that hatchery steelhead smolts are not significant predators on their natural-origin counterparts at either the smolt or pre-migrant life stage. It does not appear that predation by hatchery smolts would contribute to a reduction in natural-origin juvenile mykiss production in freshwater.

Despite this finding, there is evidence that salmonids are capable of preying on fish that are up to approximately 50% of their body length, but the majority of prey is usually much smaller. Keeley and Grant (2001) found that for 100–200 mm salmonids the typical prey size is between 13–15% of predator body size. The hatchery smolts examined in our study averaged 180mm while the natural-origin smolt present during the same sampling period ranged from 150-240mm in 2009 making them too large to prey upon. Pre-migrant mykiss juveniles falling into the optimal prey size range are ubiquitous throughout the Skagit basin (Lowery 2013). Although found throughout the basin in the spring temporal period these potential prey were not consumed suggesting that they are in someway unavailable as prey. Most hatchery smolts passed through the freshwater portion of their outmigration within 2-3 weeks. The narrow window of the hatchery outmigration period further reduces potential hatchery smolt predation encounters with sub-migrant sized mykiss. Assuming pre-migrant juvenile mykiss fall into the prey size range, it is possible that the habitat occupancy of these pre-migrants during the hatchery smolt migration period (May) may exclude them from the predation zone of hatchery smolts. Other salmonid juveniles such as pink, chum and federally listed Chinook fry were encountered and preyed upon in moderate to high numbers. This finding is to be expected since these prey species are also actively outmigrating unlike the pre-migrant steelhead juveniles. In particular, pink fry were observed to be a key prey species during even year spring migration periods. Their size, ubiquitous availability and high densities relative to other salmonid juveniles contributed greatly to their high frequency of predation.

Although not part of this study, hatchery smolts that choose not to outmigrate immediately or residualize may encounter and consume mykiss juveniles depending on location and duration of their extended freshwater rearing period.

6.0 Adult Natural-Origin Spawn Lifestage Timing and Behavior Patterns in Riverine, Estuarine and Marine Habitats

6.1 Introduction

The evaluation of possible ecological and genetic interactions between hatchery and natural-origin steelhead in the Skagit watershed necessitated a more complete understanding of the natural-origin steelhead during their adult freshwater lifestage. We attempted to learn more about the mechanisms of potential ecological or genetic intersection of these conspecifics based on behavioral patterns observed during riverine, estuarine and marine occupancy of natural-origin adults.

The spawning lifestage of adult natural-origin steelhead includes several sub-periods that are described as pre-spawn upstream migration, spawning, and a post-spawn period that spans riverine-estuarine and marine zones. The observed timing, and behavior patterns for each of these sub-periods were evaluated using bio-telemetry techniques. The data derived from this was also used to determine the spawning distribution of Skagit River natural-origin steelhead adults. Behavior patterns observed during these spawning sub-periods were identified using the acoustical data derived from this effort. It should also be noted that acoustical data were used to identify genetic baseline samples for the middle Skagit adult spawner collection (further discussed in section 8). Specifically, the following were evaluated from the acoustical dataset:

- Spatial distribution of spawners by basin or sub-reach
- Temporal effects on spawner distribution
- Pre-spawn migration patterns and behavior
- Spawning sub-period behavior patterns
- Post-spawning movement and timing

It was the opinion of the study team that the use of an acoustical tracking system would be the most effective means of collecting time/location data needed to evaluate the adult lifestage of natural-origin steelhead. Acoustical technology had previously been used successfully to collect similar data for bull trout, chum salmon, sea-run cutthroat, and steelhead juveniles within the Skagit basin. To confirm the effectiveness of this approach a pilot study was completed in 2008 to test the effectiveness of oral tag deployment and detection efficiency with adult steelhead in the Skagit watershed.

6.2 Methods

6. 2. 1 General Methods Description

A pilot study was successfully completed in 2008 that confirmed the effectiveness of oral tag deployment and detection efficiency for adult steelhead in the Skagit watershed. This was followed by three spring sampling periods from 2009-11, wherein angled adult steelhead were acoustically tagged within the middle Skagit River reach from Hamilton (RM 43.5) upstream to Concrete (RM 56). Tagging effort was spread over a five month (January-May) time period each year. Tagged fish were released at the point of capture and acoustically tracked with a receiver array spanning the Skagit watershed, Skagit Bay, Whidbey basin, Strait of Juan de Fuca and Pacific shelf (POST).

The time/location data used in this evaluation were derived from a total of 133 adult steelhead that were tagged and tracked between 2008 and 2011 (Table 9). During 2009 and 2010 tagging was spread over a 20-week time period spanning the return timing of natural-origin steelhead in the Skagit (Table 9). There was no attempt to deploy tags evenly based on gender. Females received 58% of the tags (Table 10). The average size of fish tagged varied little by year (Table 11). The adult steelhead tagged in 2010 averaged 1.5 inches less than those tagged in 2008-09. Because of this there was no effort to deploy tags by size ranges.

Table 9. Acoustic tags deployed by month in natural-origin adult steelhead during return years 2008-2011

Return Year	January	February	March	April	May	Total
2008	-	-	-	10	-	10
2009	-	2	20	14	2	38
2010	1	9	36	34	2	82
2011	1	-	1	1	-	3
Total	2	11	57	59	4	133

Table 10. Gender of tagged adult steelhead 2008-2011

Return Year	Female	Male
2008	6	4
2009	24	14
2010	46	36
2011	1	2
Total	77	56

Table 11. Average length of tagged adult steelhead 2008-2011

Return Year	N	Average Size (inches)
2008	10	29
2009	38	29.5
2010	80	27.9

The following data types were collected from each adult steelhead;

- Tagging date
- Tagging location
- DNA tissue sample (upper lobe of caudal fin)
- Scale sample (between lateral line and dorsal fin; 4/side)
- Fish length (total)
- Gender
- Tag ID number

6. 2. 2 Tag Deployment

Tags were deployed using the gastric insertion technique. This technique passes the tag through the esophagus into the stomach cavity. A specially designed tag plunger was used to pass the tag through the esophagus and into the stomach cavity. This technique was selected because it required no surgery or anesthesia and minimal handling. After a short post-tag recovery period each fish was released at the point of capture.

VEMCO Model V16-4H ultrasonic acoustic transmitters (169 db) were used throughout the study. Tag dimensions were 16x75 mm with rounded edge to ease oral insertion. Each tag weighed 12 grams in water. Regurgitation was minimized by securing a small piece of open cell foam to the external shell of each tag with a rubber band. A pulse interval 30-79 seconds was used with an expected battery life of approximately 1 year. Detection range varied with each receiver site, however most were equivalent to channel width at each location. In some locations receivers were paired to provide directionality or to increase detection probability. Overall, 89% of tagged adult steelhead between 2008-11 were detected in either the freshwater or marine portion of the array. In-river detection rates were similar with 88% of the fish detected at least once in 2009 and 94% in 2010.

6. 2. 3 Receiver Array

Adult steelhead tracking utilized the same receiver array described earlier for smolt tracking (see section 4.3.1). As was the case for smolt, the riverine deployment locations were chosen to monitor migration pathways throughout the freshwater portion of the watershed and between three defined study reaches; middle Skagit, upper Skagit, and Sauk (see Figure 2).

Receivers were deployed throughout the Skagit basin. Twenty-seven receivers were placed in mainstem channel of the Skagit River between River miles 0.0 and 97. Another 7 receiver stations were established on tributaries; Sauk River (3), Suiattle (1), Illabot Creek (1), Cascade River (1), Bacon Creek (1). Another 18 receivers at 13 stations were located downstream of the capture/tag reach in the lower mainstem and tidal delta zone. An additional set of receivers were sited within Whidbey basin and receiver lines were established near Admiralty Island and the Strait of Juan de Fuca. This array collected tag detection information that we used to determine location and movement data as accurately as possible in riverine, estuarine and marine areas. Each receiver collected fish tag code, date and time whenever a tag “ping” was detected. Receivers were downloaded periodically throughout the study period.

6. 2. 4 Data Interpretation

Raw detection data were filtered to remove data accumulated from other researchers tag series followed by the removal of false positive detections for this study's tag series. A Microsoft Access database of tag detections was then compiled. The database consisted of time and location where an individual tag was detected. The database was queried to examine the following for each fish:

- spawn location
- entry timing (tagging date) vs. spawn location
- arrival month at spawn location
- travel time to spawn location
- time spent at spawn location
- pre- and post spawn wandering
- time spent in freshwater
- post spawn outmigration timing
- marine entry timing of kelts
- Puget Sound residency time of kelts (outmigrating post-spawn steelhead)
- Freshwater/estuarine/marine pathways

6.3 Pre- Through Post Spawning Results

The results are presented in sequence by lifestage sub-period beginning with upstream migration of pre-spawn adults through outmigration of adult kelts as they migrate through riverine, estuarine and marine zones.

6.3.1 Time Needed To Reach Spawning Location

The time needed for steelhead to travel upstream to their spawning location from the middle Skagit tagging area averaged 25.4 days. However, when the location data was displayed by tagging month a strong correlation appeared (Figure 9). The earlier the tagging month (arrival month) the more time was spent arriving at spawning location with the exception of the middle Skagit river reach.

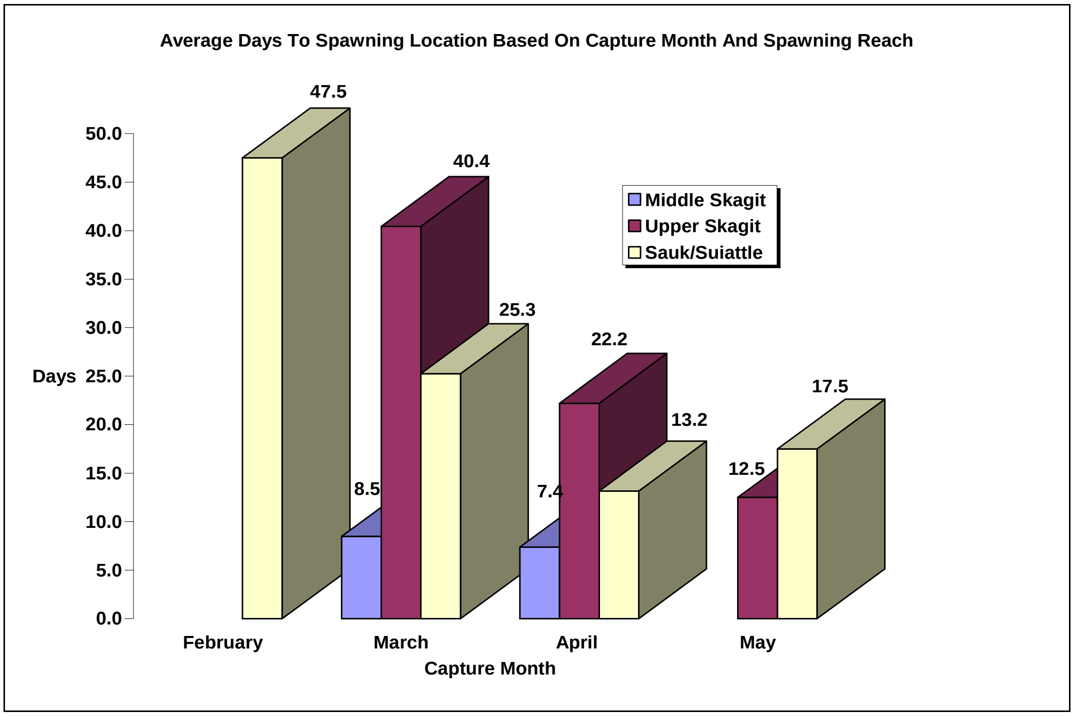


Figure 9. Average days to spawning location by natural origin steelhead based on capture (tag) month and spawning reach.

6.3.2 Factors Affecting Time Needed To Reach Spawning Location

Fish gender and size were not factors in how much time was spent reaching spawning location (Tables 12 and 13). The one possible exception was that Sauk/Suiattle male steelhead took consistently less time to reach their spawning area than females.

Table 12. Average days spent to reach spawning reach displayed by gender.

Capture Month	Middle Skagit Male	Middle Skagit Female	Upper Skagit Male	Upper Skagit Female	Sauk/Suiattle Male	Sauk/Suiattle Female
February	-	-	-	-	35.0	85.0
March	7.3	10.5	50.0	34.0	19.2	31.3
April	8.9	5.4	19.7	25.5	12.0	14.3
May	-	-	-	12.5	-	17.5

Table 13. Average days spent reaching spawning reach displayed by fish size.

Steelhead Length (inches)	N	Days To Spawning Location
21-29	79	25.2
30-37	34	26.0

6.3.3 Spawning Location Determination

Not all acoustically tagged adult steelhead could be tracked to spawning location. However, the presumptive spawning location was determined for 87% of the 130 fish tagged during 2008-10 study period. Spawning locations were aggregated into three large sub-reaches; middle Skagit, upper Skagit and Sauk/Suiattle. For all years the highest proportion of spawning occurred in the most downstream middle Skagit reach followed by a roughly equal split between the upper Skagit and Sauk/Suiattle reaches (Table 14). Within each sub-reach there were reach segments that consistently tallied the highest number of spawners each year. For the middle Skagit reach the reach segment between Birdsview and Dalles Bridge was the highest for all but 2008. The upper Skagit reach segment with highest total for all three years was from Rockport to Marblemount. In the Sauk reach the segment above Darrington attracted the highest number of spawners for all years. There was no significant difference in the percentage of steelhead spawning in the three reaches for years 2009 and 2010. Most fish arrived at spawning locations in April and May. The middle Skagit reach adult steelhead genetic baseline sample collection was identified using acoustic spawning location results.

Table 14. Presumptive spawning locations of adult steelhead by year based on acoustical tracking data. Numbers in parenthesis are sample size within each of the three defined river reaches.

River Reach	2008	2009	2010	All Years
Middle Skagit (below Sauk confluence)	100% (7)	48% (14)	55% (42)	56% (63)
Upper Skagit (Above Sauk Confluence)	0%	35% (10)	21% (16)	23% (26)
Sauk/Suiattle Rivers	0%	17% (5)	25% (19)	21% (24)

6.3.4 Influence of Capture Month on Spawn Location

Most of the early arrival (February/March) natural-origin steelhead spawned in either the middle Skagit or the Sauk/Suiattle locations (Figure 10). Fish arriving in the month of May, the latest arriving adults, spawned primarily in the most upstream reaches of the watershed; upper Skagit and Sauk/Suiattle. Very few of these late arrival fish spawned in the middle Skagit reach.

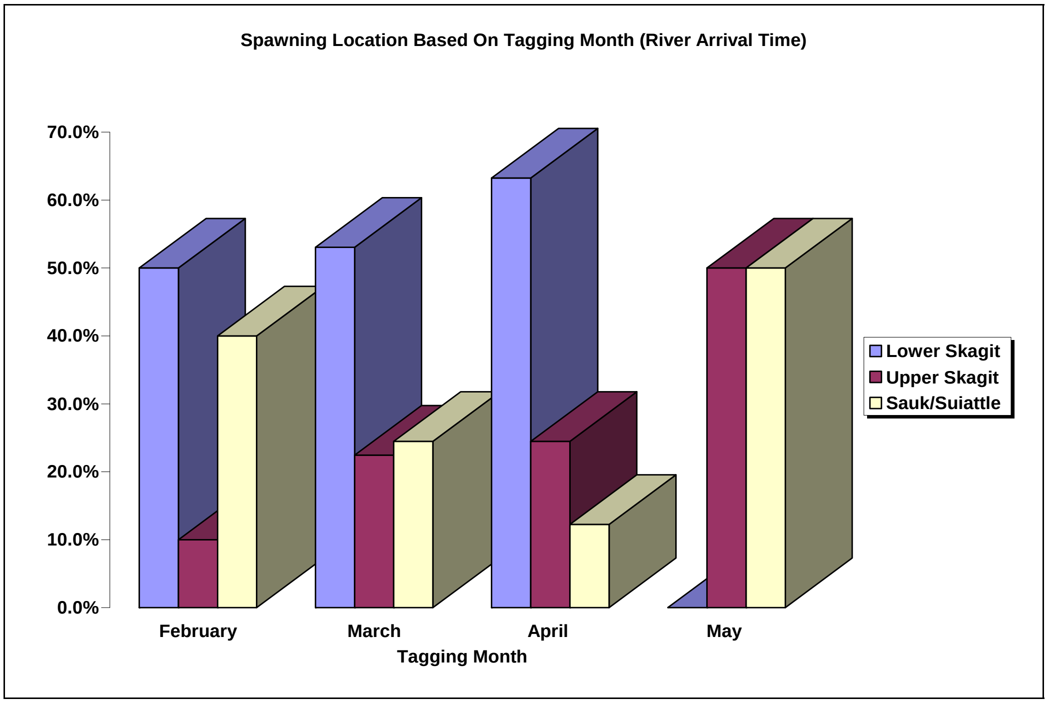


Figure 10. Spawning location based on tagging month (river arrival time)

6.3.5 Affect of Arrival Month on Spawn Location

There were few differences in spawning location based on the month when fish arrived at their spawning reach (Figure 11). Overall the preponderance of fish arrives during the March-May time period, while smaller percentages arrive during the months of January, February and June. A large portion of the lower Skagit reach adults arrive in the February-April time period as compared to the other spawning reaches. Upper Skagit adults tended to arrive later than for the other two spawning reaches.

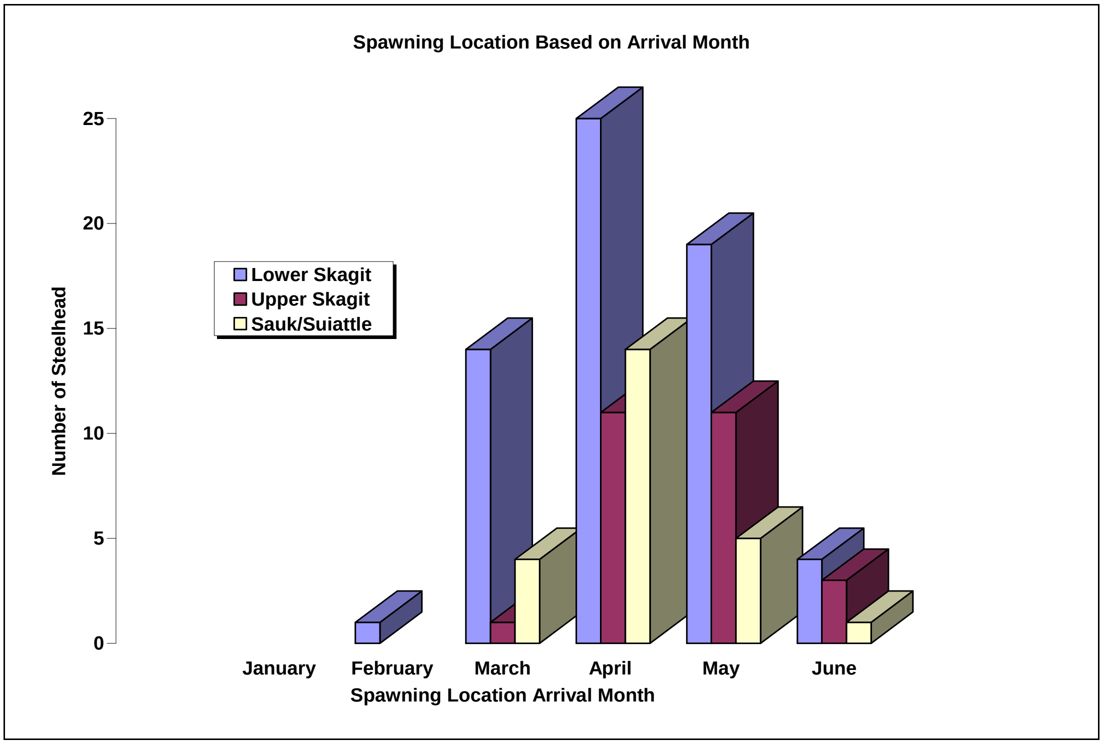


Figure 11. Differences in spawning location based on arrival month.

6.3.6 Time Spent At Spawning Location

The time spent at all three spawning locations decreased with later capture month. Later arriving fish spent less time at the spawning location than those arriving during earlier months (Figure 12).

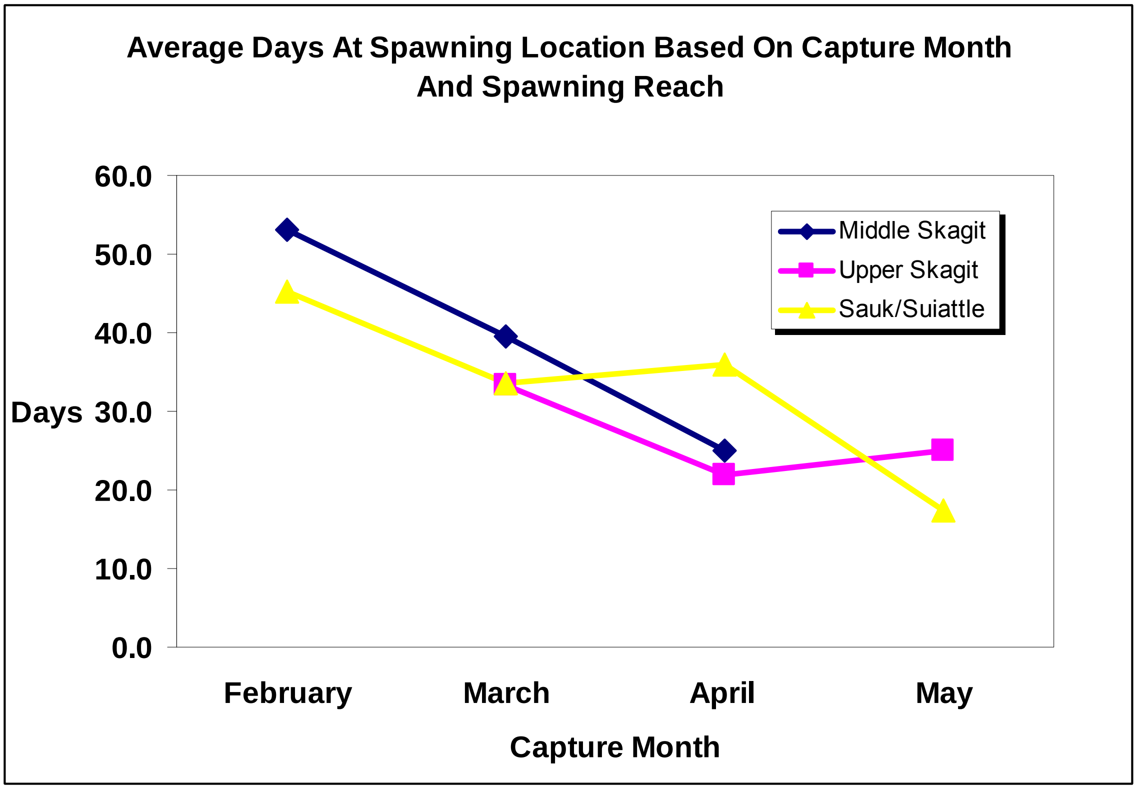


Figure 12. Average days at spawning location determined from capture month and spawning reach.

6.3.7 Pre- and Post-Spawn Wandering

Nine out of 113 (9.2%) tracked fish showed some form of wandering or straying during pre-spawn, spawn or post-spawn periods. Removing the two post-spawn fish reduces wandering/stray rate to 6.1%. More females demonstrated wander tendencies than males; 7 females vs. 2 males (two females wandered post-spawn).

6.3.8 Post-Spawn Outmigration

The time taken by kelt steelhead to outmigrate from their individual spawning locations to marine influenced waters varied greatly. The average travel time was 14.5 days when including a single fish that took 255 days to outmigrate (Figure 13). Removal of this outlier from the sample of 69 total kelts tracked reduced the average to 11 days.

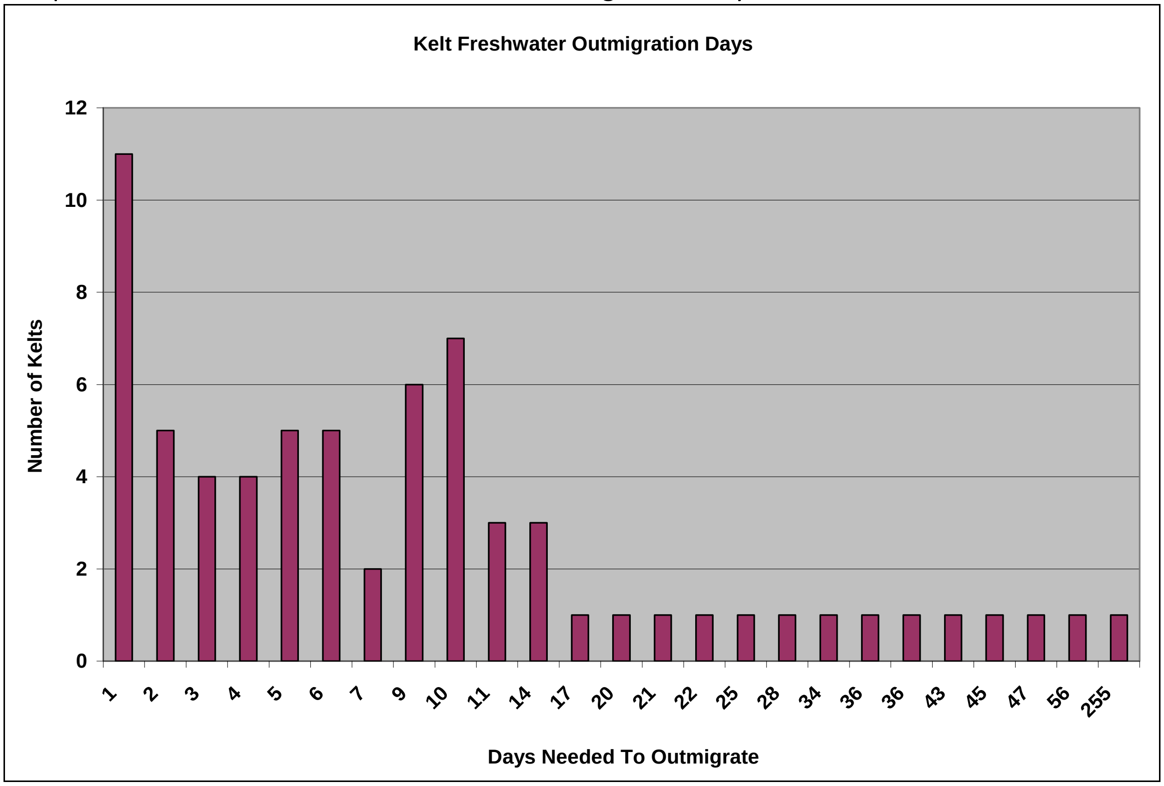


Figure 13. Days kelt steelhead spent outmigrating to marine waters.

6.3.9 Time Spent In Freshwater By Adults

Regardless of which spawning reach was used by individual fish, natural-origin steelhead spent between 63-71 days in freshwater from the time they were tagged in the middle Skagit reach to when kelts entered Whidbey basin (Figure 14). The longest and shortest freshwater adult residency times varied little between spawning locations. The average male freshwater residency time was nearly a month longer than what was observed for females (Figure 15). The longest freshwater residency observed was slightly over 9-months in duration for a male and nearly 5 ½ months for a female.

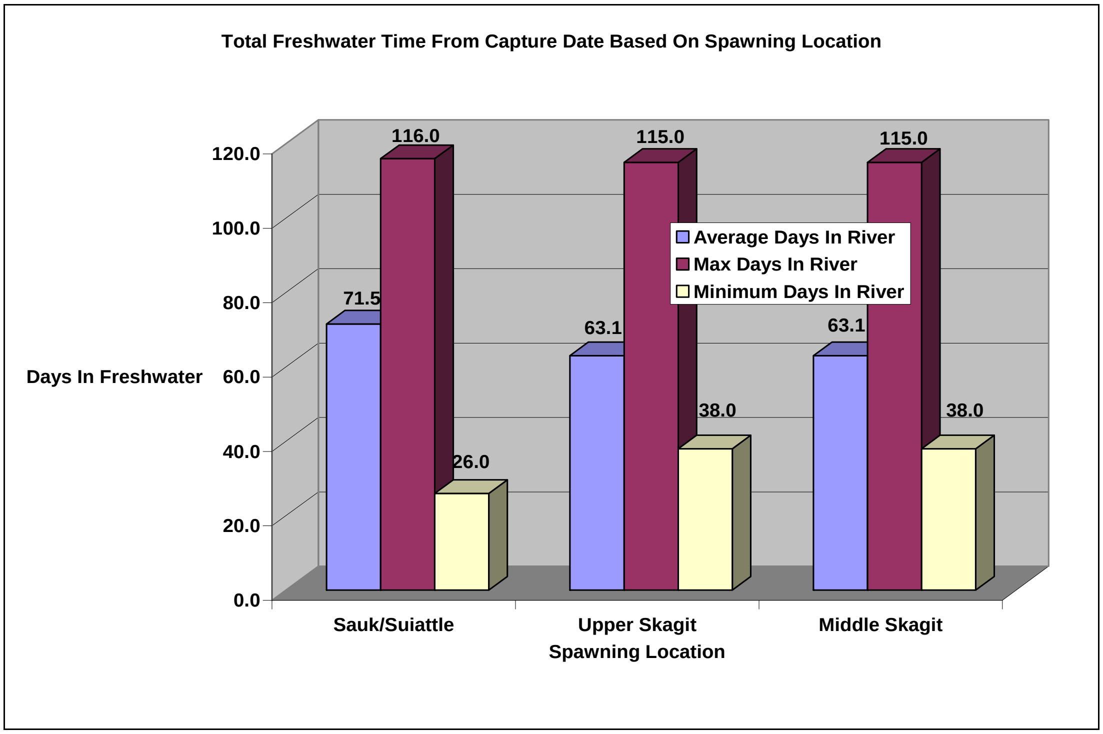


Figure 14. Time spent in freshwater during the spawning period for fish from three spawning locations.

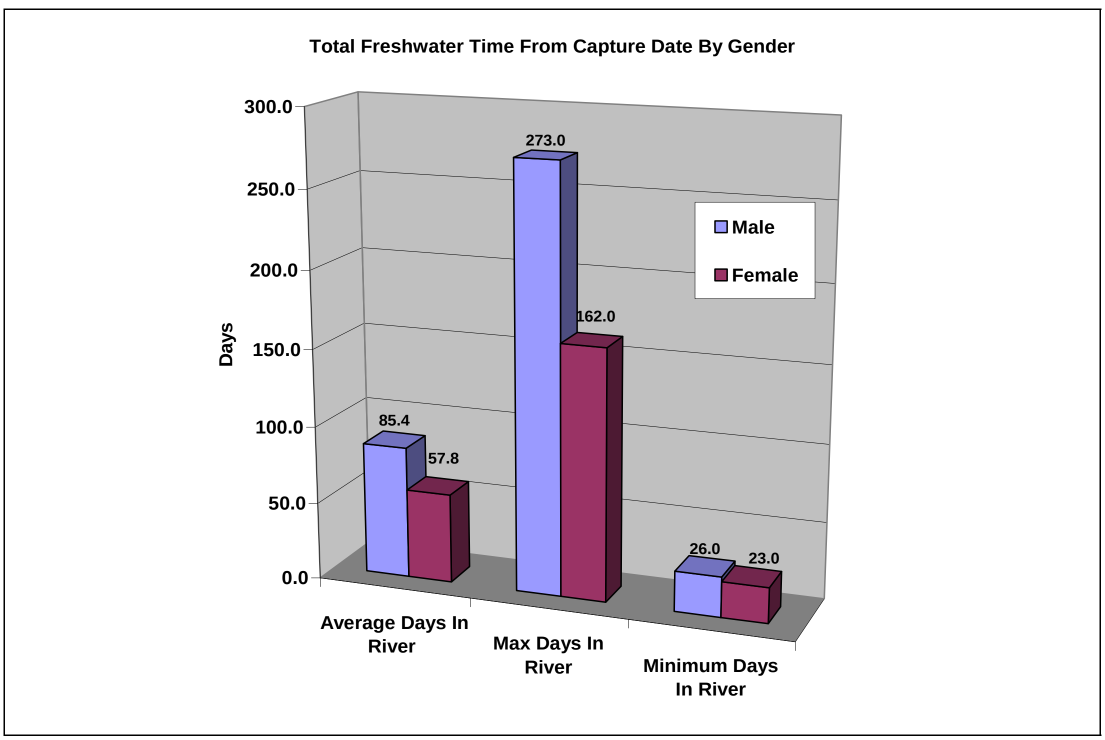


Figure 15. Time spent in freshwater during the spawning period based on fish gender.

6.3.10 Marine Entry Timing of Kelts

Most kelt steelhead arrived in tidal or marine waters during the months of May and June (Figure 16). The earliest observed kelt arrived in March and the latest entered marine waters in December many months after spawning.

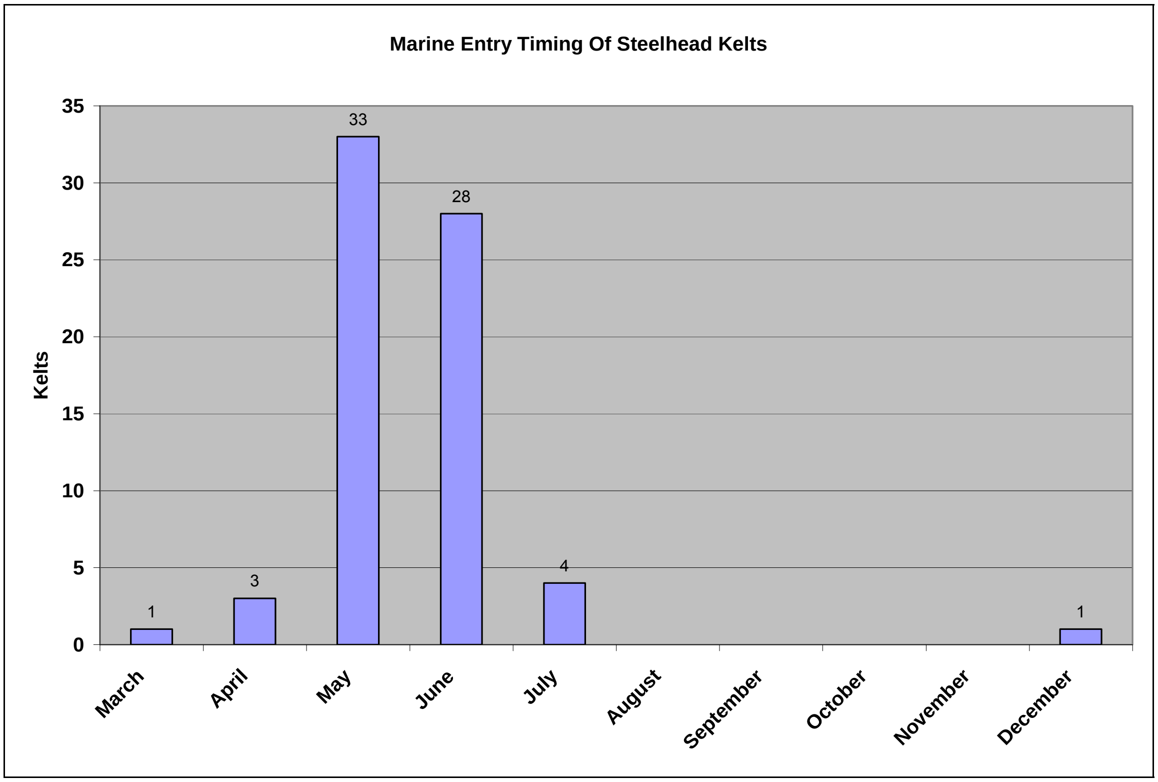


Figure 16. Marine entry timing of kelts.

6.3.11 Kelt Residency Time in Puget Sound

Eighteen kelts were tracked across the Strait of Juan de Fuca (SJF) receiver line (Figure 17). Twelve of these travelled from the Skagit River tidal-marine area past the SJF line within three days. The remaining individuals remained in Puget Sound from 13-70 days before passing the receiver line at the SJF.

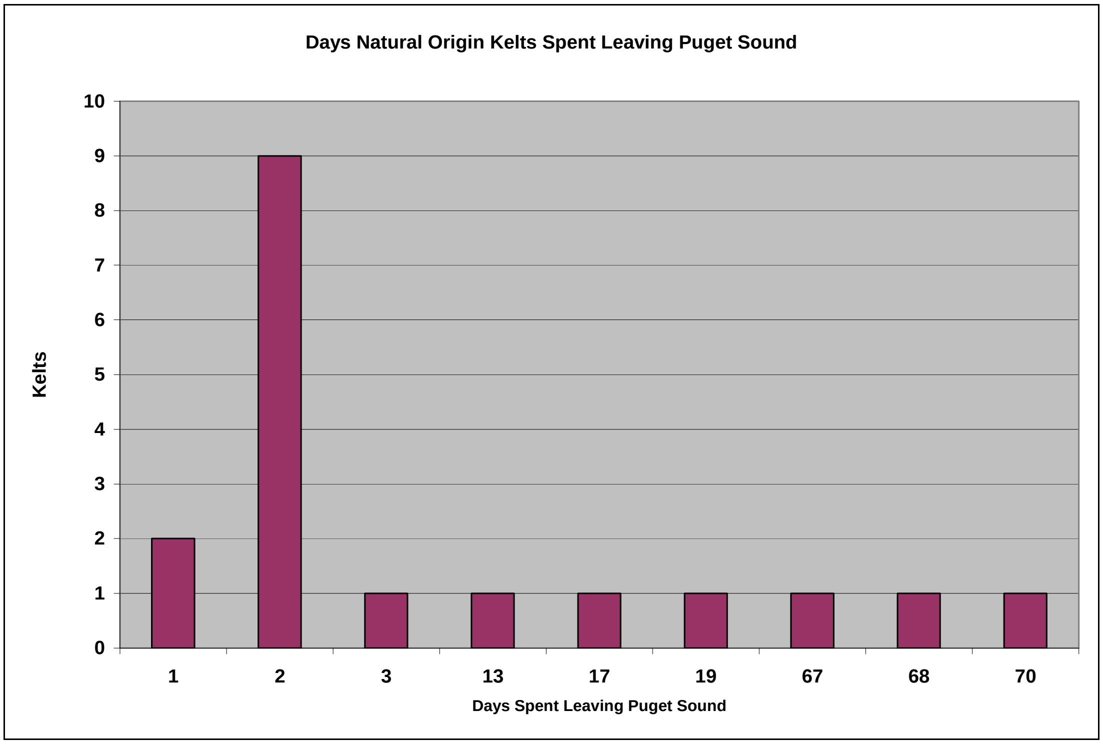


Figure 17. Kelt residency time in Puget Sound.

6.4 Discussion and Conclusions

To understand where areas of ecological or genetic overlap exist between hatchery and natural-origin steelhead adults a more complete understanding of each conspecific during the adult freshwater spawning lifestage is required. Possible mechanisms of potential ecological or genetic intersection were evaluated for several spawning lifestage sub-periods. The spawning sub-periods are described as pre-spawn upstream migration, spawning, and a post-spawn period that spanned riverine- estuarine and marine zones.

Our observations were limited to natural-origin steelhead adults. Similar information about Marblemount hatchery steelhead could not be collected as part of this study and does not exist from other sources. In the absence of this information we make the assumption that hatchery adult behavior is similar to that of natural-origin fish during the spawning life stage. More specifically, natural spawn hatchery steelhead likely demonstrate the same behavioral capability regarding wandering tendencies, upstream migration travel and time spent in their spawning location.

Salmonids, such as steelhead, exhibit a level of fidelity by returning to their natal stream to reproduce. A component of these adults is also known to stray and reproduce outside of their natal stream (Schroeder 2001). The level of straying can vary from a different stream reach, sub-basin or an entirely different watershed. Schroeder found that in Oregon coastal streams the percentage of strays averaged 11% (range 4-26%) of the samples of hatchery and wild fish in 11 streams where hatchery steelhead were released. Stray hatchery fish composed a mean of 22% (range 9-43%) in 5 streams without hatchery releases. He found that the two predominant factors that contributed to straying were releases of stocks transplanted from their natal basins and releases into adjacent basins. Releases of transplanted stocks into adjacent basins accounted for 41% of the strays, while releases of transplanted stocks into nonadjacent basins accounted for 29% of the strays. Local stocks of steelhead released into adjacent basins accounted for 16% of the strays. The incidence of straying by hatchery fish and its widespread occurrence in Oregon coastal rivers was shown to present genetic and ecological risks to wild populations of winter steelhead.

Indecision about spawning location can also take the form of wandering or taking an indirect route to the spawning location. In the case of a natural spawn hatchery steelhead derived from the integrated Marblemount program this would be any hatchery adult spawning outside the hatchery. By tracking the upstream pathway of natural-origin adults we were able to document any wandering tendencies observed. Of those successfully tracked to their spawning location, 6.1% showed some form of wandering while reaching their spawning location. Clearly, this is was a relatively small portion of those tracked and it may be that others in the tracked group took a direct route even though they may have strayed from what would have otherwise been their natal spawning area. Of those that did wander, it is not possible to determine if these fish were exhibiting low fidelity and straying to a location other than their natal area. Our data also showed that more females than males showed a wandering tendency.

For genetic hybridization to occur hatchery and natural-origin adults must both be present and actively spawning. Telemetry results showed that natural-origin steelhead spent an average of 63-71 days in freshwater before returning downstream to marine areas. There were individuals that remained in fresh water for up to 116 days. Males remained in fresh water longer than females, averaging 85 days with one individual remaining for 273 days. If hatchery adults exhibit the same freshwater duration capability then hybridization interactions would need to occur during the 2-3 month period of freshwater occupancy. Given the early return timing of Chambers Creek origin hatchery adults, the most likely hybridization interactions would be between late arriving hatchery fish and natural-origin individuals spawning on the early side of their typical spawn period.

The data suggests that this type of overlap is possible. Both spawned and unspawned hatchery adults are present and documented in March for many years (see section 7). Assuming stray hatchery steelhead can spend 2-3 months in freshwater like natural-origin fish then hybridization is possible during the months of March and April especially with any early spawning natural-origin steelhead such as are found most commonly in the middle Skagit reach and tributaries. Our telemetry results show that most of the earliest arriving natural-origin fish are from the middle Skagit reach. WDFW personnel further support our telemetry data by adding that their spawning surveys typically find that the earliest natural-origin spawners are seen in middle Skagit tributaries such as Finney and Grandy creeks (Brett Barkdull, 2011, personal communication). Given this, it would be expected that the frequency of hybridization would be highest where the most spawn timing overlap occurs between these conspecifics.

The extent of hybridization is thought to be governed by several factors; the size of the hatchery release and associated survival to adulthood, hatchery stray rate, return timing and overlap with actively spawning natural-origin adults. Our data suggests that opportunities exist for stray hatchery-origin adults to encounter natural-origin fish throughout the basin but most frequently in areas where spawning activity occurs in March and April such as in the middle Skagit reach and its tributaries.

7.0 Evidence of Hatchery Straying and Natural Spawning Within the Skagit Watershed from Adult Capture Data Sources

7.1 Introduction

A primary objective of a segregated steelhead hatchery program is to prevent reproductive and ecological interactions between natural-origin and hatchery populations. For the Marblemount hatchery a key operating goal is for all hatchery steelhead adults to either return to the hatchery or be caught in a tribal or sport fishery. An important element of this study was to collect data capable of evaluating whether this goal is being met.

Ecological and genetic impacts resulting from hatchery steelhead spawning outside of the hatchery have been shown by other researchers to compromise overall productivity of natural-origin populations (Christie et al. 2012, Kostow 2009, Seamons 2011). Christie and Seamons demonstrated that the progeny resulting from a natural-origin – hatchery cross have a greatly reduced level of reproductive success. This is an example of genetic introgression that leads to compromised survival of natural-origin steelhead. Another type of interaction that does not fall into the category of genetic introgression occurs when steelhead of hatchery-origin spawn with each other (hatchery x hatchery mating) outside of the hatchery. The resulting hatchery-origin progeny will occupy habitat causing possible ecological implications. The final level of genetic interaction considered results from hatchery-natural-origin hybrids returning as an adult to spawn with a natural-origin adult resulting in reduced reproductive success (Christy 2012).

7.2 Methods

While direct observations of Marblemount hatchery steelhead adults spawning outside of the hatchery is difficult and limited, other more indirect data were used to document whether some level of hatchery steelhead fail to return to the hatchery and attempt to reproduce within the Skagit basin. Between 2008 and 2012, angling and tribal fishery data were used to provide evidence of natural spawning by Marblemount hatchery adults. In addition, available data from angled steelhead were used to identify the spatial range, timing and general abundance of hatchery stray spawners. Angling data used for this evaluation included information on capture location, date, gender and whether each fish was unspawned or a kelt. Tribal fishery data provided information on hatchery kelt numbers and timing. Collectively these data were used to establish the presence, relative abundance and timing of natural spawning hatchery steelhead within the Skagit watershed.

7.3 Results

Unspawned and kelt hatchery-origin steelhead captured outside the hatchery after March 1 for each return year are shown in Figure 18. Hatchery steelhead shown in this figure had either spawned outside the hatchery or were captured after the established time frame for spawning at the Marblemount hatchery. Stray hatchery adults, both spawned and un-spawned, have been collected or observed in the mainstem Skagit, Sauk and Cascade rivers as well as in tributaries such as several middle Skagit reach tributaries including Savage, Finney, Mill creeks.

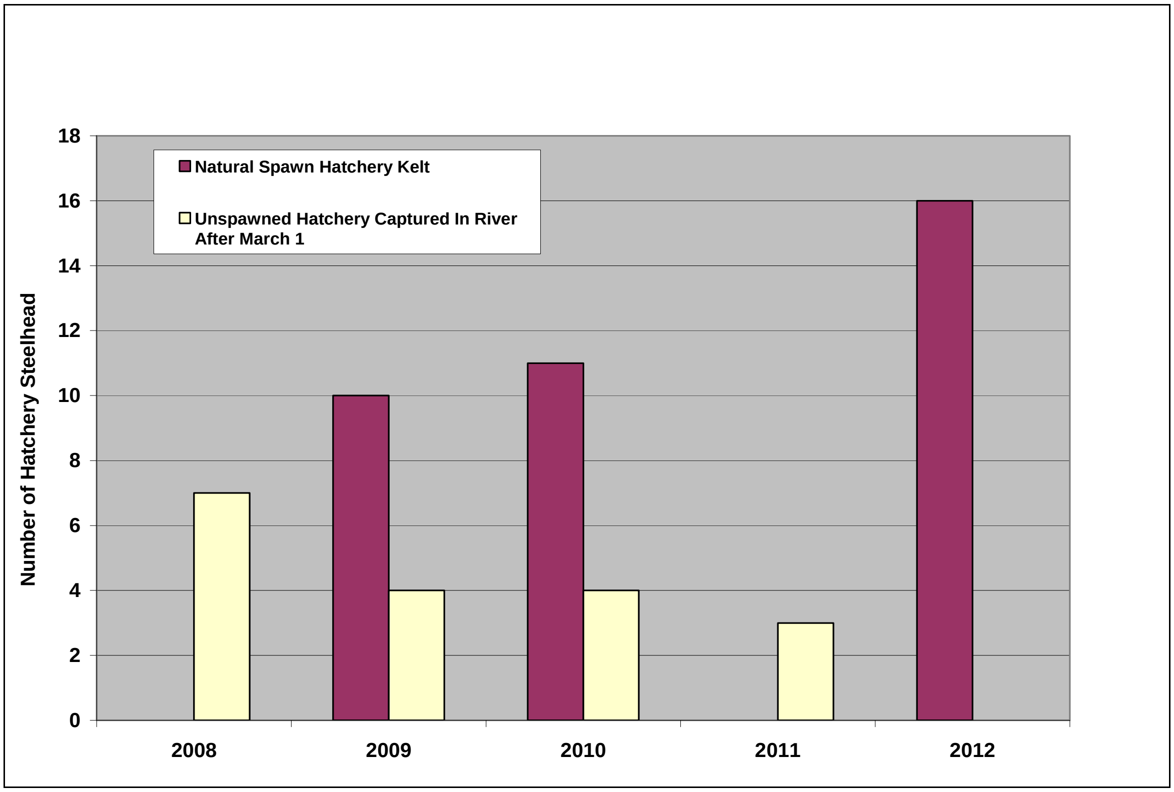


Figure 18. Hatchery steelhead kelts spawning outside the Marblemount hatchery and unspawned hatchery steelhead captured in-river after March 1 by year from 2008-2012.

WDFW scale interpretation information was also used to show evidence of hatchery steelhead repeat spawning from adults captured in a tribal fishery (Figure 19). In most years between 2005 and 2011 there were examples of hatchery steelhead having spawned multiple times based on scale interpretation. Hatchery steelhead that do return to the Marblemount hatchery are spawned a single time and killed preventing repeat spawning. These data provide evidence showing that hatchery-origin steelhead strays are capable of spawning multiple times outside of the hatchery.

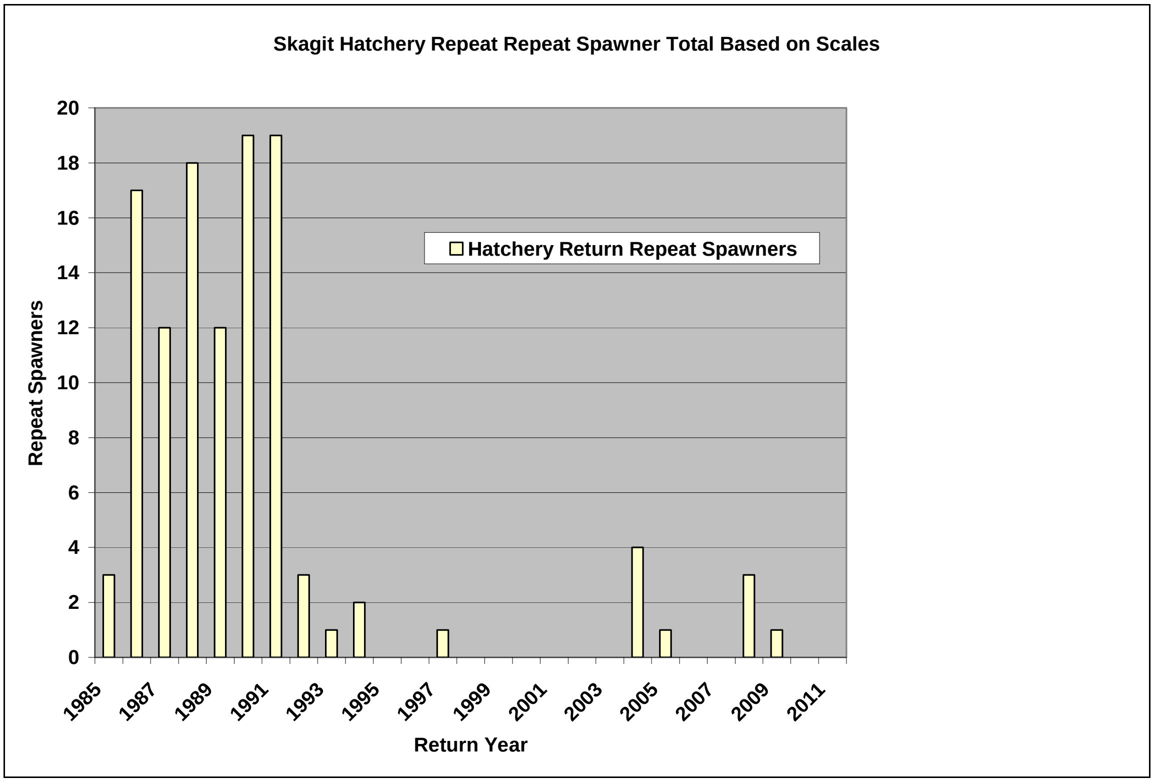


Figure 19. Hatchery Steelhead identified as repeat natural spawners based on scale patterns (Scale interpretation by WDFW).

7.4 Discussion/Conclusion

Genetic and ecological interactions between natural and hatchery-origin steelhead can only occur if hatchery adults become strays instead of returning to the hatchery or hatchery smolts choose the stream-type life history. For reproductive interactions to take place hatchery stray fish need to be present in natural spawning areas when natural are also present. Without this spawn-time overlap hybridization is not possible.

The capture of both spawned and unspawned hatchery-origin steelhead at a variety of mainstem and tributary locations verified the occurrence of straying throughout the Skagit watershed. This finding confirms that there is opportunity for genetic and ecological interactions with natural-origin steelhead. Furthermore, it was established that a number of stray hatchery adults are returning after February which is far later than desired for the Marblemount segregated hatchery program. These fish overlap with the spawn timing of natural-origin steelhead throughout the basin creating opportunities for reproductive hybridization. This is especially true for earliest spawning natural-origin steelhead typically found in the middle Skagit mainstem and its tributaries.

Our results as well as findings from other researchers found that late returning hatchery-origin adults, especially males, on the Skagit were found to stay in fresh water for many months (Leider et al. 1984; Seamons et al. 2004). Both studies found that hatchery males in particular are capable of remaining in fresh water until natural-origin females arrive and mate with wild fish throughout the wild spawning season, thus producing offspring with relatively late return timing. On the Skagit it appears that the largest overlap in spawn timing occurs in the middle

Skagit reach, especially in the tributaries where some of the earliest natural-origin spawning takes place.

Based on scale interpretation, hatchery strays have also been shown to be capable of repeat spawning outside of the hatchery. Multiple reproductive cycles by a number of strays further extends the potential amount of genetic and ecological interaction with their natural-origin counterparts.

The degree to which hatchery-origin steelhead stray and residualize in the Skagit remains unclear. However, it is likely that it varies annually depending on several factors such as number of smolt released, smolt to adult survival and freshwater flow conditions during adult upstream migration.

8.0 Population Structure Within the Skagit Based on Genetics

8.1 Introduction

Starting March 2008, caudal fin tissue was collected from hatchery and natural-origin steelhead adults and juveniles for use in extracting DNA (Table 15). Similar samples were also taken from four populations of resident rainbow trout residing above migrational barriers located on Finney Creek, Clear Creek (Upper Sauk basin), Big Creek (Upper Suiattle River) and North Fork Cascade River. Additional resident rainbow trout samples were acquired from several tributaries or reservoirs located upstream of the Skagit River Hydroelectric project (Table 7). Because of past stocking introductions into Ross reservoir by British Columbia (BC) an additional collection was derived from one out-of-basin population on the Blackwater River (BC) rainbow trout. DNA samples were also taken from the caudal fins of adult hatchery steelhead that had returned to the Marblemount hatchery in return years 2008-2010 (Table 15). Also, because of its proximity to the Skagit River, a final hatchery baseline was established from samples obtained from the Chilliwack River Hatchery in British Columbia. These samples were used to form DNA baselines for the 14 collection areas or types.

Table 15. DNA baseline sampling collections

DNA Baseline Groups	Steelhead Adults	Steelhead Juveniles	Resident Rainbow Trout*
Marblemount Hatchery	✓		
Upper Skagit River Mainstem	✓	✓	
Chilliwack Hatchery (BC)	✓		
Middle Skagit River Mainstem	✓	✓	
Goodell Creek		✓	
Cascade River	✓	✓	✓
Upper Baker River		✓	
Finney Creek	✓	✓	✓
Sauk River	✓	✓	✓
Suiattle River	✓	✓	✓
Ross Lake Rainbow			✓
Stetattle Creek (Diablo Lake)			✓
Blackwater River BC (Non-Anad/Juv)			✓
Sauk River (1981)	✓		
Sauk River (1983)	✓		

*Rainbow trout populations located above anadromous barriers

All samples were preserved and transported to the WDFW genetics lab in Olympia, Washington for processing. Previously collected (archived) adult steelhead tissue samples from the Sauk River in the 1980s were also included in this genetic evaluation to determine possible time-series shifts in hatchery-wild origin hybridization levels and ancestry.

There was one previous effort to genetically characterize steelhead populations in the Skagit basin at a spatial level. It was conducted in 1979 by U. S. Fish and Wildlife Service and Washington State Department of Game. The three year study was terminated after a single year but did provide some coarse information on the genetic structure of natural-origin Skagit and British Columbia and Chambers Creek and Skamania hatchery stock steelhead (USFWS 1980).

For this study, genetic analysis techniques were used to provide information about basic genetic characteristics of natural and hatchery-origin steelhead populations and resident mykiss populations. Other analytic methods were used to evaluate ancestry, hybridization level and introgression in natural-origin adult and juvenile steelhead collections on a spatial level. Lastly, juvenile and adult ancestry data were used to identify where natural-spawning hatchery steelhead appear to be reproducing successfully.

Steelhead populations in the Skagit River basin are being supplemented by the production of hatchery steelhead at Marblemount Hatchery that provide for the harvest of steelhead within the basin. Marblemount Hatchery, which is owned and operated by WDFW, is the main hatchery in the Skagit River. It is located near the town of Marblemount on the Cascade River. The hatchery complex also includes Barnaby Slough; a rearing pond for winter steelhead located at RM 70.2 on the Skagit River downstream of Marblemount Hatchery near the town of Rockport. The average 1995–2005 brood year (BY) releases from Marblemount Hatchery complex were 413,900 with a maximum release in 2000 of 663,470 smolts and pre-smolts. The average BY releases from 1985–1995 were 286,337 smolts and pre-smolts. All fish are currently 100% marked with an adipose fin clip and released at 6 fish per pound in May. Steelhead adult collection and spawning takes place from late December to March.

However, the impacts of hatchery steelhead that are from out of basin (Chambers Creek origin) on natural populations are unknown, but hatchery fish are speculated to have lower fitness than wild fish (Araki et al. 2008). They have also suggested that the fitness of a non local stock is lower than a stock that was taken from the local source are better, but still lower than the wild fish.

This project is specifically intended to examine the genetic impacts of hatchery steelhead on wild stocks, by examining the extent of interbreeding, and whether interbreeding produces persisting hatchery genes in wild stocks. By answering these questions, hatchery steelhead programs can be modified to reduce these impacts, which should result in increased abundance of the natural steelhead stocks

Management in the Skagit Basin is primarily directed towards the need of natural production; however, the steelhead hatchery program on the Skagit has a harvest-oriented purpose. The hatchery program uses a segregated strategy incorporating the Chambers origin steelhead stock which is earlier return timed than the Skagit wild steelhead stock.

The objectives of this study are:

- Analyze natural-origin steelhead adults and juveniles from mainstem reaches and tributaries within the Skagit River basin and address if steelhead within and among each reach and tributary are genetically homogeneous or differentiated?

- Analyze steelhead from Marblemount Hatchery and Chilliwack Hatchery, B.C. to assess if they are differentiated to Skagit natural-origin steelhead

- Analyze resident rainbow trout from non anadromous areas within the Skagit River basin and compare them to natural-origin juvenile and adult steelhead.

- Analyze natural-origin steelhead adults, juveniles, and resident rainbow trout within the same basin to determine if there has been downstream migration of the resident trout mixing with the anadromous steelhead.

- Analyze steelhead collected in the Sauk River from the 1980's and 2010's to determine if there has been a change in the genetic profile of natural-origin steelhead over time.

- Analyze steelhead harvested in fisheries to determine the stock composition of the catch.

- Assessment of natural and hatchery-origin steelhead to determine if there is any evidence of genetic introgression.

8.2 Methods

8.2.1 Collections

A total of 3,079 fish were sampled from mainstem reaches and tributaries of the Skagit River basin from 2008 – 2010 including collections from the Sauk River that were made in the 1980's (Table 16). Attempts were made to collect samples from 100 adult steelhead, 100 juvenile steelhead from anadromous zones, and 100 resident rainbow trout above barriers from each sampling location. Temporal collections were combined to increase sample sizes when there were not enough samples collected in a given year. These baseline collections of natural-origin steelhead were made in 12 different locations within anadromous zones in the Skagit River basin, five locations of resident *O.mykiss* from the upper Skagit River, and one location of resident *O.mykiss* from British Columbia. Samples were also collected from five different fisheries. Lastly, collections from Marblemount Hatchery and Chilliwack River Hatchery, B.C. broodstock were collected. The Chilliwack River Hatchery program is distinguished from Marblemount because it is an "integrated" program that uses natural-origin broodstock unlike the segregated program at Marblemount that was initiated from Chambers Creek (Puget Sound) broodstock. The Marblemount Hatchery "early-timed" collection (10KA; Table 16) included adult steelhead that returned to the hatchery from late September through October, which was considerably earlier than normal return timing of November through February; we speculated that these fish might be summer-run steelhead from another facility.

Table 16. Collections of anadromous adults, juveniles, and resident *O.mykiss* from the Skagit River basin. Aggregate group number represents collections that could be combined into a group because they were not genetically distinct from other temporal collections from the same location. A different aggregate group number from the same location identifies temporal collections that were genetically differentiated and analyzed separately. Collections with samples sizes of less than five that were analyzed were dropped from analyses (highlighted in grey).

Aggregate Group #	WDFW Code	Collection Location	life stage	N collected / analyzed
1	08DQ	upper Skagit River	adults	20 / 13
1	09BN	upper Skagit River	adults	10 / 10
1	10AO	upper Skagit River	adults	25 / 24
1	11BI	upper Skagit River	adults	34 / 34
2	07MT	upper Skagit River	juveniles	13 / 10
x	08MJ	upper Skagit River	juveniles	3 / 3
2	10AZ	upper Skagit River	juveniles	60 / 58
x	08LU	middle Skagit River mainstem	adults	3 / 1
4	09BM	middle Skagit River mainstem	adults	43 / 11
4	10AS	middle Skagit River mainstem	adults	31 / 31
3	10LG	mid Skagit River - 1/4 pounders	adults	10 / 10
5	09EI	lower Skagit River (Hamilton area)	juveniles	27 / 19
6	10AY	lower Skagit River (Hamilton area)	juveniles	50 / 48
x	08MI	Bacon Creek	adults	4 / 4
18	07MS	Bacon Creek	juveniles	9 / 7
x	09IX	Bacon Creek	juveniles	27 / 0
18	10BA	Bacon Creek	juveniles	50 / 50
x	09DS	Cascade River	adults	2 / 2
x	10AP	Cascade River	adults	2 / 2
21	09EE	lower Cascade River	juveniles	52 / 49
22	10AV	lower Cascade River	juveniles	50 / 49
24	09ES	N.F. Cascade River	RBT	50 / 49
24	10BF	N.F. Cascade River	RBT	50 / 49
x	81AAB	Suiattle River	adults	2 / 2
x	09DT	Suiattle River	adults	1 / 1
7	10AQ	Suiattle River	adults	17 / 17
7	11BM	Suiattle River	adults	34 / 34
x	09EF	Suiattle River	juveniles	5 / 4
8	10AW	Suiattle River	juveniles	61 / 60
25	09EU	Big Creek (trib to Suiattle River)	RBT	54 / 46
26	10BG	Big Creek (trib to Suiattle River)	RBT	50 / 50
9	81AAA	Sauk River	adults	37 / 37
9	83AAA	Sauk River	adults	30 / 30
9	08DR	Sauk River	adults	13 / 10
9	08MS	Sauk River	adults	8 / 8

Aggregate Group #	WDFW Code	Collection Location	life stage	N collected / analyzed
9	09DU	Sauk River	adults	17 / 16
9	10AR	Sauk River	adults	24 / 23
9	11BN	Sauk River	adults	24 / 24
x	09EG	Sauk River	juveniles	5 / 3
10	10AX	Sauk River	juveniles	50 / 50
27	09ET	Clear Creek (trib to Sauk River)	RBT	53 / 50
27	10BE	Clear Creek (trib to Sauk River)	RBT	50 / 48
37	09EL	Baker River - upstream of Baker Lake	RBT	65 / 41
38	10AU	Baker River - upstream of Baker Lake	RBT	50 / 33
x	08MT	Finney Creek	adults	2 / 2
11	10CQ	Finney Creek	adults	23 / 23
11	11BK	Finney Creek	adults	30 / 30
12	09EH	Finney Creek	juveniles	65 / 54
13	10AT	Finney Creek	juveniles	51 / 51
28	09EV	upper Finney Creek	RBT	53 / 37
29	10BD	upper Finney Creek	RBT	50 / 48
17	07MU	County Line Ponds	juveniles	11 / 8
17	08MK	County Line Ponds	juveniles	6 / 6
17	09IY	County Line Ponds	juveniles	27 / 18
17	10BB	County Line Ponds	juveniles	52 / 42
19	09IZ	Goodell Creek	juveniles	49 / 43
20	10BC	Goodell Creek	juveniles	50 / 45
x	09LM	Diobsud Creek	juveniles	9 / 0
23	10BK	Diobsud Creek	juveniles	50 / 47
14	08LF	Marblemount Hatchery	adults	46 / 39
14	09CF	Marblemount Hatchery	adults	69 / 65
14	10AN	Marblemount Hatchery	adults	50 / 47
15	10KA	Marblemount Hatchery (early timed)	adults	18 / 18
16	10MZ	Chilliwack River (Hatchery broodstock)	adults	74 / 71
		Upper Skagit River - Resident RBT Collections and Collection from B.C.		
30	06AF	Ross Lake	RBT	83 / 48
31	09MA	Ross Lake	RBT	50 / 40
32	10BH	Ross Lake	RBT	50 / 47
33	03OA	Dry Creek	RBT	54 / 50
x	04AAB	Dry Creek	RBT	25 / 0
33	04AAD	Dry Creek	RBT	12 / 12
34	02FB	Roland Creek	RBT	100 / 97
35	05NG	Diablo Lake	RBT	117 / 62
36	09JA	Stetattle Creek	RBT	36 / 33

Aggregate Group #	WDFW Code	Collection Location	life stage	N collected / analyzed
36	10BI	Stetattle Creek	RBT	50 / 43
39	09JB	Blackwater River, B.C. (tributary to Fraser River)	RBT juv	18 / 18
39	10BJ	Blackwater River, B.C. (tributary to Fraser River)	RBT juv	50 / 49
		Skagit River Fishery Collections		
x	94AAA	Skagit River Fishery - unclipped steelhead	adults	42 / 42
x	08MR	Skagit River Fishery - unclipped steelhead	adults	128 / 124
x	08MU	Skagit River Fishery - clipped steelhead	adults	10 / 10
x	09JC	middle Skagit River Fishery - unknown clips	adults	103 / 97
x	10AE	middle Skagit River Tribal Fishery - clipped and unclipped steelhead	adults	92 / 72
			Total	3,079 / 2,658

8.2.2 Laboratory Analyses

All laboratory analyses were conducted at the WDFW Genetics Laboratory in Olympia, Washington. Genomic DNA was extracted by digesting a small piece of tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the manufactures recommended protocol.

The standardized suite of 15 microsatellite markers for analyses of steelhead were screened (SPAN dataset; Stephenson et al. 2009) using standard laboratory protocols and analysis methods at WDFW. Descriptions of the loci assessed in this study and polymerase chain reaction (PCR) conditions are given in Table 17. PCR reactions were run with a simple thermal profile consisting of: denaturation at 95°C for 3 min, denaturation at 95°C for 15 sec, anneal for 30 sec at the appropriate temperature for each locus, extension at 72°C for 1 min, repeat cycle (steps 2-4), final extension at 72°C for 30 minutes. PCR products were then processed with an ABI-3730 DNA Analyzer. Genotypes were visualized with a known size standard (GS500LIZ 3730) using GENEMAPPER 3.7 software. Allele calls followed standardized allele sizes established for the Steelhead SPAN dataset (Stephenson et al. 2009).

Table 17. PCR conditions and microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used for the analysis of O.mykiss. Also included are the observed and expected heterozygosity (H_o and H_e) for each locus.

PCR Conditions				Locus statistics		Heterozygosity		
Poolplex	Locus	Dye Label	Annealing temp (^{O}C)	# Alleles/ Locus	Allele Size Range (bp)	H_o	H_e	References
Omy-L	One-102	6fam	47	24	184 - 277	0.8154	0.8924	Olsen et al. 2000
	Oke-4	hex	47	17	234 - 272	0.6991	0.7957	Buchholz et al. 2001
	Ots-100	ned	47	29	166 - 232	0.8088	0.9017	Nelson and Beacham 19
Omy-M	Oki-23	6fam	55	23	112 - 200	0.8612	0.9182	Smith et al. 1998
	Omy-7	hex	55	21	234 - 278	0.7496	0.8431	K. Gharbi, pers. comm.
	Ssa-408	pet	55	22	173 - 265	0.8304	0.9055	Cairney et al. 2000
Omy-N	Ots-4	6fam	59	11	105 - 129	0.7342	0.8015	Banks et al. 1999
	Omy-1011	ned	59	26	118 - 246	0.8442	0.9179	Spies et al. 2005
Omy-O	Omy-1001	6fam	49	30	168 - 246	0.7717	0.9002	Spies et al. 2005
	Ots-3M	ned	49	12	68 - 102	0.5730	0.6595	Banks et al. 1999
Omy-P	Ssa-407	6fam	59	27	151 - 263	0.8085	0.9023	Cairney et al. 2000
	Ogo-4	hex	59	15	115 - 143	0.6858	0.7662	Olsen et al. 1998
	One-14	pet	59	13	146 - 208	0.5700	0.7361	Scribner et al. 1996
Omy-Q	Ssa-289	hex	50	13	99 - 123	0.4837	0.5730	McConnell et al. 1995
	Oki-10	none	50	25	121 - 201	0.6822	0.7794	Smith et al. 1998

8.2.3 Statistical Analyses – Population Statistics

Hardy-Weinberg proportions and genotypic differentiation for all loci within each collection were calculated using GENEPOP (version 3.4, Raymond and Rousset 1995) to determine if the temporal collections from the same location could be combined. Statistical significance for the tests of Hardy-Weinberg proportions and genotypic differentiation was evaluated using a Bonferroni correction of p-values to account for multiple, simultaneous tests (Rice 1989). Our expectation or null hypothesis was that fish sampled in the same location among years would be genetically similar (non-significant temporal variation) due to homing behavior and gene flow among brood years (multiple spawner age classes)

Temporal collections that were not significantly different were combined for all further analyses. Allele frequencies for loci and combined temporal collections were calculated with CONVERT (version 1.3, Glaubitz 2003). Heterozygosity (observed and expected) was computed for each collection group using GDA (Lewis and Zaykin 2001).

Allelic richness and F_{IS} (Weir and Cockerham 1984) inbreeding coefficient were calculated using FSTAT (version 2.9.3.2, Goudet 2001). Linkage disequilibrium for each pair of loci in each collection was calculated using GENEPOP v 3.4 (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Statistical significance for the tests of Hardy-Weinberg proportions, linkage disequilibrium, and genotypic differentiation was again evaluated using a Bonferroni correction of p-values to account for multiple, simultaneous tests (Rice 1989).

8.2.4 Statistical Analyses – Differences Among Groups

Pairwise estimates of genotypic differentiation between collection groups were calculated using GENEPOP (version 3.4, Raymond and Rousset 1995) by calculating the differences in allele frequencies for all loci analyzed. Significance of the genotypic differentiation test is determined by the adjusted p-value using a Bonferroni correction. A value that is lower than the adjusted p-value for the comparison between two collections is significantly different. Pairwise F_{ST} estimates were also computed to examine differentiation among collections or collection groups using a permutation test in GENETIX (version 4.03, Belkhir et al. 2001). This estimate uses allelic frequency data and departures from expected heterozygosity to assess differences between pairs. The closer the pairwise comparison is to 1.0 identifies collections that are completely different and do not have any common alleles while values of 0.0 identify collections with no differentiation. Comparisons were made by evaluating each pairwise F_{ST} value and whether it was significantly different from zero.

GENETIX (version 4.03, Belkhir et al. 2001) was used for a factorial correspondence analysis and a graphical representation of the genetic variation among all individual samples in multi-dimensional space. Genotypic data for an individual sample is transformed into a value and plotted. The multi-dimensional data space represents all the individual values. Each axis (three-dimensional in this case) is derived from the individual values that correspond to percent of total chi-square distance, with chi-square measuring the association between individual genotypes and allele frequencies.

The number of genetically distinguishable groups and ancestry for steelhead individuals in the Skagit River basin were evaluated using a Bayesian analysis implemented in the program STRUCTURE 2.1 (Pritchard et al. 2000). Structure uses an iterative process to partition the dataset into a number of genetic clusters (populations) which minimizes Hardy-Weinberg disequilibrium and linkage disequilibrium in the clusters. Three independent runs were conducted allowing admixture with 50,000 burn-ins and 450,000 iterations. Analyses were conducted using all individuals with K (number of possible groups or populations) set from 1 to 10. At K = 1, we were testing the hypothesis that the samples were from a single ancestral group, with K = 2, we were testing whether natural-origin Skagit and Marblemount hatchery steelhead were from genetically distinguishable groups, and with K = 3+ we were testing for genetic distinction among populations of steelhead and rainbow trout collected from different tributaries in the Skagit River basin.

8.2.5 Stock of Origin Analysis of Fisheries

We used the program ONCOR (Kalinowski, downloaded January 2008 from http://www.montana.edu/kalinowski/Software/ONCOR.htm) to perform 100% simulations using the Realistic Fishery Simulation model by Anderson et al. (2008) on the baseline collections. We ran 100 percent simulations for each of the baseline populations to determine the probability of assignment of individuals to each of the baseline populations (see Table 16). The number of iterations was set at 100 with a mixture size of 200 individuals per collection. The results identify the average value with the 95% confidence interval surrounding the average value for the individual baseline collections.

We also used ONCOR to calculate the estimated mixture proportions of baseline collections among steelhead captured during the fishery. ONCOR uses conditional maximum likelihood to estimate mixture proportions (Millar 1987) and genotype probabilities are calculated using the method of Rannala and Mountain (1997).

8.3 Results

8.3.1 Microsatellite DNA Locus Statistics

Tests of Hardy-Weinberg (H-W) equilibrium for each locus and collection were evaluated for any significant deviation after Bonferroni correction (Rice 1989). All adult collections were in Hardy-Weinberg equilibrium while four collections of juveniles (upper Skagit River – *Omy-1001*, lower Skagit River – *Omy-1001*, Goodell Creek – *One-102*, and Cascade River – *Omy-7*) each had one locus that was not in H-W equilibrium. Eight collections of rainbow trout had loci that did not meet Hardy-Weinberg expectation; however there were only two loci for three collections and one locus for five different collections that were not in H-W equilibrium. All collections were included in further analyses because the collections and loci not in equilibrium were limited and did not suggest there had been non-random mating of individuals (inbreeding or assortative mating) in the collection.

8.3.2 Population Level Statistics – Genotypic Differentiation Among Temporal Collections

Collections of adult and juvenile steelhead and resident rainbow trout were analyzed to determine if temporal collections from the same locations were genetically differentiated and should be analyzed as distinct. Collections with small sample sizes were also excluded. A total of 30 adult steelhead collections were analyzed for temporal variability, and based on findings of non-significance collections from the same locality were combined into nine aggregate collection locations for the analysis of population structure. A total of 22 juvenile steelhead collections were analyzed and temporal collections with non-significant differences were aggregated, resulting in 14 aggregated or original collections for further analyses. A total of 21 collections of resident rainbow trout were analyzed and aggregated into 16 distinct groups.

8.3.3 Population Level Statistics

The population level statistics (Hardy-Weinberg equilibrium and F_{IS} inbreeding coefficient) were consistent with neutral expectations (i.e., no associations among alleles) with exception of eight aggregate collections with significant deviations from Hardy-Weinberg expectations and four rainbow trout collections (Clear Creek, Diablo Lake, Baker River 2010, and Blackwater River) that had significant F_{IS} values after Bonferroni correction. The eight collections with significant Hardy-Weinberg values had fewer than two loci that did not meet expectations; therefore those collections did not represent groups of non-randomly mating individuals and were included with further analysis. The significant F_{IS} values at four rainbow trout collections are likely the result of closely related individuals being sampled.

Average heterozygosity for the aggregated groups of adults and juvenile steelhead collections was 0.7807 and 0.7821 respectively ranging from 0.7533 – 0.8199 (Table 18). The value for the hatchery collections was slightly lower at 0.7714 (range 0.7498 – 0.7906) and the collections of rainbow trout had the lowest average heterozygosity at 0.6672 (range 0.3707 – 0.7417). The collection of rainbow trout from the Cascade River had the lowest value at 0.3707. Without the Cascade River collection the heterozygosity in the rainbow trout collections was still lower than that of the natural and hatchery-origin steelhead collection aggregates with an average of 0.68045 (range 0.6203 – 0.7417). Heterozygosity is a measure of the molecular variation at a given locus or in a collection. This level of variation is utilized in statistical analyses to determine if the variation meets the expected values in Hardy Weinberg proportion to describe the population and locus. A collection with a small sample size or from a group with relatively few breeding individuals may have a lower heterozygosity value as seen in the rainbow trout collection from the Cascade River.

Allelic richness values for the aggregated groups of collections revealed the highest average value in the collections of adult steelhead (7.4) and lowest in the collections of rainbow trout (5.6) based on sample size of 10 diploid individuals. The average allelic richness in the juvenile and hatchery-origin collections was slightly lower than observed in the adult collections (7.1 and 6.7 respectively). Allelic richness like heterozygosity is a measure of the genetic diversity detected in a population sample and is affected by the number of individuals contributing to that population (e.g. populations with few individuals or populations with related individuals will have lower allelic richness values).

Linkage disequilibrium among loci was highest in the Marblemount Hatchery collections with 65 – 37 significant comparisons before and after Bonferroni correction. The collections of juveniles from Finney Creek and Cascade River also had over 50 significant comparisons before correction. A large number of significant comparisons can be caused by genetic drift of small populations, presence of related individuals in the collection, a collection that includes a mixture of genetically different individuals, and/or mutation or selection.

Table 18. Collections of O.mykiss taken in the Skagit River basin with the collections parsed into aggregate groups and collection statistics (allelic richness, F_{IS} (p-value), Heterozygosity (H_E and H_O), and linkage disequilibrium).

	Aggregate Group #	Allelic Richness[a]	F_{IS} (p-value)[b]	H_e	H_o	Linkage Dis[c]
up Skagit River adults	1	7.6	0.012 (0.1643)	0.7993	0.7896	9 / 1
up Skagit River juveniles	2	7.3	0.022 (0.0674)	0.7890	0.7721	21 / 3
mid Skagit River 1/4 pounders	3	7.1	0.033 (0.2240)	0.7779	0.7533	2 / 0
mid Skagit River adults	4	7.5	0.027 (0.0656)	0.7958	0.7749	4 / 0
lower Skagit River juveniles '09	5	6.9	-0.021 (0.7926)	0.7773	0.7935	14 / 2
lower Skagit River juveniles '10	6	7.2	0.021 (0.1003)	0.7926	0.7761	26 / 6
Suiattle River adults	7	7.4	0.004 (0.3846)	0.7839	0.7806	10 / 0
Suiattle River juveniles	8	7.4	0.004 (0.3750)	0.7945	0.7912	8 / 1
Sauk River adults	9	7.3	0.033 (0.0006)	0.7925	0.7661	30 / 8
Sauk River juveniles	10	7.4	0.036 (0.0168)	0.7993	0.7707	10 / 2
Finney Creek adults	11	7.7	-0.014 (0.8226)	0.8083	0.8199	4 / 0
Finney Creek juveniles '09	12	7.1	-0.003 (0.5771)	0.7826	0.7852	51 / 11
Finney Creek juveniles '10	13	7.3	-0.001 (0.5206)	0.7976	0.7986	10 / 1
Marblemount Hatchery	14	7.2	0.016 (0.0438)	0.8034	0.7906	65 / 37
Marblemount early	15	6.5	0.005 (0.4206)	0.7777	0.7739	5 / 0
Chilliwack Hatchery	16	6.3	0.011 (0.2396)	0.7581	0.7498	5 / 0
County Line Pounds juveniles	17	7.2	0.021 (0.0647)	0.7904	0.7736	26 / 7
Bacon Creek juveniles	18	7.2	0.01 (0.2605)	0.7908	0.7828	14 / 2
Goodell Creek juveniles '09	19	6.8	0.012 (0.2618)	0.7730	0.7638	31 / 9
Goodell Creek juveniles '10	20	7.0	-0.011 (0.7274)	0.7848	0.7935	24 / 2
Cascade River juveniles '09	21	6.3	-0.037 (0.9745)	0.7670	0.7947	50 / 19
Cascade River juveniles '10	22	7.0	0.027 (0.0642)	0.7871	0.7663	56 / 18
Diobsud Creek juveniles	23	7.4	0.01 (0.2777)	0.7950	0.7871	22 / 2
Cascade River RBT	24	3.3	0.041 (0.0190)	0.3863	0.3707	3 / 0
Big Creek RBT '09	25	4.5	0.035 (0.0648)	0.6496	0.6274	6 / 0
Big Creek RBT '10	26	4.5	0.066 (0.0020)	0.6637	0.6203	29 / 4
Clear Creek RBT	27	6.4	0.064 (0.0001)	0.6845	0.6406	20 / 5
Finney Creek RBT '09	28	5.4	0.056 (0.0062)	0.7323	0.6918	14 / 0
Finney Creek RBT '10	29	5.5	0.035 (0.0377)	0.7133	0.6884	10 / 0
Ross Lake RBT '06	30	6.4	0.037 (0.0326)	0.7199	0.6938	13 / 2

	Aggregate Group #	Allelic Richness[a]	F_{IS} (p-value)[b]	H_e	H_o	Linkage Dis[c]
Ross Lake RBT '09	31	5.5	-0.003 (0.5591)	0.6839	0.6860	31 / 4
Ross Lake RBT '10	32	5.3	-0.017 (0.7948)	0.6960	0.7076	28 / 5
Dry Creek RBT	33	5.9	0.016 (0.1700)	0.7140	0.7027	26 / 4
Roland Creek RBT	34	5.9	0.001 (0.4546)	0.7031	0.7021	14 / 0
Diablo Lake RBT	35	6.2	0.077 (0.0001)	0.7371	0.6810	5 / 0
Stetattle Creek RBT	36	6.6	0.031 (0.0140)	0.7655	0.7417	19 / 1
Baker River RBT '09	37	7.1	0.065 (0.0004)	0.7844	0.7343	29 / 5
Baker River RBT '10	38	7.5	0.105 (0.0001)	0.8083	0.7245	16 / 2
Blackwater River, B.C. RBT	39	6.4	0.114 (0.0001)	0.7470	0.6624	8 / 2

a - based on 10 haploid individuals; b - adjusted alpha p-value - 0.0001; c - adjusted alpha p-value 0.0004

8.3.4 Genetic Differentiation Among Groups

The genotypic differentiation and pairwise F_{ST} analysis of the nine aggregate collections of adult steelhead revealed that all of the natural-origin collections were not significantly differentiated (Table 19). The highest pairwise F_{ST} value for the natural-origin adult collections occurred between Finney Creek and middle Skagit River quarter pounders (0.0076), all other values were below 0.0039.

Table 19. Pairwise F_{ST} values (above diagonal) and genotypic differentiation (below diagonal) for adult Skagit steelhead. Values in bold identify pairwise comparisons that are not significantly different from zero and genotypic differentiation values that were not significantly different.

	upper Skagit River	mid Skagit River 1/4 pounders	mid Skagit River	Suiattle River	Sauk River	Finney Creek	Marblemount Hatchery	Marblemount early	Chilliwack Hatchery
mid Skagit River 1/4 pounders	**0.2053**	****	**0.0031**	**0.0039**	**0.0035**	**0.0076**	0.0227	**0.0341**	**0.0315**
mid Skagit River	**0.3133**	**0.3567**	****	**-0.0012**	**0.0004**	**0.0006**	0.0230	0.0346	0.0161
Suiattle River	**0.2208**	**0.3786**	**0.3267**	****	**-0.0006**	**0.0039**	0.0254	0.0299	0.0189
Sauk River	**0.2471**	**0.2411**	**0.1653**	**0.8389**	****	**0.0011**	0.0263	0.0302	0.0176
Finney Creek	**0.2627**	**0.0266**	**0.7066**	**0.0146**	**0.2210**	****	0.0199	0.0248	0.0222
Marblemount Hatchery	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	**0.0100**	0.0460
Marblemount early	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0567
Chilliwack Hatchery	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****

Analysis of the 14 aggregate and non-aggregated juvenile collections identified significant genotypic differences between all collections with the exception the Goodell Creek 2010 and County Line Ponds collections (Table 20). The pairwise F_{ST} comparisons however were significantly different from zero at over half the comparisons. There were no observable patterns to the significant comparisons in the temporal juvenile collections (some of the temporal collections were significant while other temporal collections were not significantly different). The difference among the juvenile collections is possible because they were collected from fewer of the overall individuals with higher relatedness (sampling siblings or family groups) in the population and therefore represent a smaller portion of the overall genetic diversity. The differences in allele frequencies among temporal collections can be significantly different while the population as a whole is not genetically differentiated.

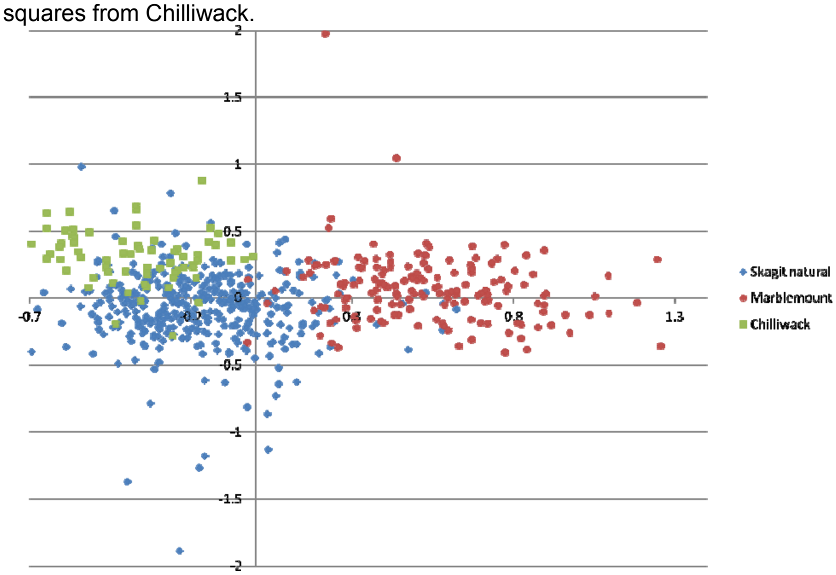
Table 20. Pairwise F_{ST} values (above diagonal) and genotypic differentiation (below diagonal) for juvenile steelhead collected in the Skagit River basin. Values in bold identify pairwise comparisons that are not significantly different from zero and genotypic differentiation values that were not significantly different.

	up Skagit Juv	low Skagit Juv '09	low Skagit Juv '10	Suiattle River Juv	Sauk River Juv	Finney Creek Juv '09	Finney Creek Juv '10	County Line Ponds Juv	Bacon Creek Juv	Goodell Creek Juv '09	Goodell Creek Juv '10	Cascade River Juv '09	Cascade River Juv '10	Diobsud Creek Juv
up Skagit Juv	****	**0.0128**	**0.0058**	**0.0047**	0.0115	**0.0061**	**0.0050**	**0.0054**	**0.0055**	0.0117	**0.0056**	0.0231	**0.0072**	0.0089
low Skagit Juv '09	0.0002	****	**0.0168**	0.0205	**0.0262**	0.0212	**0.0188**	**0.0143**	0.0245	**0.0145**	**0.0118**	0.0363	**0.0150**	0.0260
low Skagit Juv '10	0.0000	0.0000	****	0.0105	0.0207	0.0101	**0.0075**	**0.0083**	0.0129	0.0152	**0.0093**	0.0236	0.0122	**0.0102**
Suiattle River Juv	0.0000	0.0000	0.0000	****	0.0123	0.0111	**0.0056**	0.0101	**0.0083**	0.0112	**0.0087**	0.0201	**0.0055**	0.0112
Sauk River Juv	0.0000	0.0000	0.0000	0.0000	****	**0.0100**	**0.0074**	0.0123	**0.0078**	0.0173	0.0112	0.0276	0.0118	0.0117
Finney Cr. Juv '09	0.0000	0.0000	0.0000	0.0000	0.0000	****	**0.0047**	0.0127	**0.0061**	0.0158	0.0087	0.0274	0.0115	0.0092
Finney Cr. Juv '10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0098	**0.0040**	**0.0086**	**0.0059**	0.0176	**0.0059**	0.0080
County Line Ponds Juv	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0095	**0.0079**	**0.0012**	0.0257	**0.0085**	**0.0062**
Bacon Creek Juv	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0142	**0.0073**	0.0274	0.0096	**0.0039**
Goodell Cr. Juv '09	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	**0.0031**	0.0226	**0.0118**	0.0141
Goodell Cr. Juv '10	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0342**	0.0000	0.0000	****	0.0221	**0.0044**	**0.0054**

	up Skagit Juv	low Skagit Juv '09	low Skagit Juv '10	Suiattle River Juv	Sauk River Juv	Finney Creek Juv '09	Finney Creek Juv '10	County Line Ponds Juv	Bacon Creek Juv	Goodell Creek Juv '09	Goodell Creek Juv '10	Cascade River Juv '09	Cascade River Juv '10	Diobsud Creek Juv
Cascade R. Juv '09	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0190	0.0265
Cascade R. Juv '10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0110
Diobsud Creek Juv	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	****

Analysis of the natural-origin collections with the hatchery-origin revealed significant genotypic differences between the natural-origin and the three hatchery collections from Marblemount (early and normal timed) and Chilliwack. Comparison of the three hatchery collections to the natural-origin collections revealed an average pairwise F_{ST} value of 0.0244. The average pairwise F_{ST} value of the natural-origin juveniles and Marblemount Hatchery adult collections was 0.0285 while the comparison of natural-origin to Chiliwack Hatchery was slightly lower at 0.0209. The factorial correspondence plot from GENETIX shows separation in two dimensions of the three groups, all natural-origin steelhead adults, all Marblemount Hatchery samples and all Chilliwack Hatchery samples relative to each other (Figure 20). The individuals in the natural-origin and Chilliwack Hatchery collections had considerable overlap while the individuals in the Marblemount Hatchery collection had very little overlap with the Chilliwack Hatchery collection and minor overlap with natural-origin Skagit steelhead.

Figure 20. Factorial correspondence plot of natural-origin steelhead in the Skagit River Basin, Marblemount Hatchery, and Chilliwack Hatchery. Diamond shapes represent Individual samples from the natural-origin collection, circles from Marblemount and squares from Chilliwack.



The collection of early returning Marblemount Hatchery steelhead was not significantly different from the collection of normal timed steelhead from Marblemount Hatchery, but both were significantly different from steelhead from the Chilliwack Hatchery (BC) local-origin steelhead (integrated program).

Analysis of the resident rainbow trout within the Skagit River basin from Baker River, Big Creek (tributary to the Suiattle River), Cascade River, Clear Creek (tributary to the Sauk River), and Finney Creek had pair-wise F_{ST} values that were all significantly different from zero with an average pairwise F_{ST} value of 0.2110 (range of 0.0931 – 0.3840 with exception of the temporal collections from Baker River, Big Creek and Finney Creek; Table 21). A collection of resident rainbow trout from Blackwater River (British Columbia) was also analyzed and had an average pairwise F_{ST} value of 0.1557 (range of 0.0767 -0.3183) when compared to Skagit basin rainbow trout collections. Pair-wise comparison of the rainbow trout collections from the upper Skagit River (Dry Creek, Diablo Lake, Ross Lake, Roland Creek, and Stetattle Creek) to each other revealed an average pairwise F_{ST} value of 0.0260 (range 0.0052 – 0.0457), and all pair-wise values were significantly different from zero. The only exception was the pairwise F_{ST} comparison between Roland and Dry Creek (0.0052; not significantly different from zero), and genotypic differentiation between Roland and Dry Creek that was not significantly different. All other genotypic differentiation pair-wise comparisons of upper Skagit rainbow trout collections were significantly different from each other.

Table 21. Pairwise F_{ST} values (above diagonal) and genotypic differentiation (below diagonal) for resident rainbow trout. Values in bold identify pairwise comparisons that are not significantly different from zero and genotypic differentiation values that were not significantly different.

	Cas-cade River	Big Cr. '09	Big Cr. '10	Clear Cr.	Finney Cr. '09	Finney Cr. '10	Ross L. '06	Ross L. '09	Ross L. '10	Dry Cr.	Roland Cr.	Diablo L.	Stetattle Cr.	Baker R. '09	Baker R. '10	Blackwater R.
Cascade R	****	0.3840	0.3795	0.3221	0.3689	0.3583	0.3386	0.3644	0.3808	0.3384	0.3357	0.3272	0.3019	0.3055	0.3310	0.3183
Big Cr. '09	0.0000	****	0.0157	0.2314	0.2071	0.2203	0.1916	0.2079	0.2080	0.1991	0.2012	0.1696	0.1630	0.1699	0.1547	0.1820
Big Cr. '10	0.0000	0.0000	****	0.2116	0.1959	0.2077	0.1918	0.2089	0.2116	0.2004	0.2027	0.1777	0.1642	0.1667	0.1531	0.1803
Clear Cr.	0.0000	0.0000	0.0000	****	0.1633	0.1599	0.1885	0.1997	0.2003	0.1832	0.1908	0.1692	0.1512	0.0931	0.0972	0.1654
Finney Cr. '09	0.0000	0.0000	0.0000	0.0000	****	**0.0105**	0.1358	0.1559	0.1371	0.1354	0.1366	0.1212	0.1059	0.0968	0.0937	0.1123
Finney Cr. '10	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.1474	0.1627	0.1398	0.1427	0.1473	0.1329	0.1127	0.0998	0.1036	0.1299
Ross L. '06	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0238	0.0432	0.0140	0.0131	0.0254	0.0225	0.0920	0.0927	0.0758
Ross L. '09	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0415	0.0152	0.0172	0.0457	0.0289	0.1118	0.1164	0.0963
Ross L. '10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0350	0.0275	0.0412	0.0418	0.1049	0.1028	0.0790
Dry Cr.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	**0.0052**	0.0269	0.0225	0.0899	0.0928	0.0819
Roland Cr.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0093**	****	0.0204	0.0173	0.0929	0.0984	0.0834
Diablo L.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0175	0.0760	0.0789	0.0798
Stetattle Cr.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0579	0.0655	0.0690
Baker R. '09	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	**0.0076**	0.0808
Baker R. '10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0002**	****	0.0767
Blackwater R.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****

All resident rainbow trout collections analyzed were significantly different to collections of natural-original and hatchery-origin steelhead collections with the exception of the collection of resident rainbow trout from Baker River.

The STRUTURE analysis to assess the most likely number of distinct genetic groups and the genetic ancestry of the natural-origin adult steelhead and rainbow trout identified seven different genetic groups (Table 22a). Each of the collections had greater than 80% of their ancestry into one of the seven groups. The seven groups included the following collections: the upper Skagit natural-origin steelhead and Baker River rainbow trout were in group 1; rainbow trout from the Cascade River, Big Creek, Clear Creek, Finney Creek, and Blackwater Creek were each identified to a different group (groups 2, 3, 4, 5, and 7 respectively); and the seven collections of resident rainbow trout from the upper Skagit River were all in group 6. The seven collections from the upper Skagit River were then analyzed separately to determine if there was any genetic structure that was not apparent when all 14 collections were analyzed (Table 22b). The analysis of the upper Skagit River rainbow trout collections identified three genetic groups: Diablo and Stetattle were in group 1; Dry Creek and Roland Creek were in group 2; and Ross Lake 2010 was in group 3. The other two collections had ancestry that was split into two different groups: Ross Lake 2006 groups 1 and 2; Ross Lake 2009 groups 2 and 3.

Table 22a. Proportion of genetic ancestry to one of seven different groups for a collection of adult natural-origin steelhead in the Skagit River and collections of rainbow trout.

	N =	1	2	3	4	5	6	7
Skagit River natural -origin	375	**91.0%**	0.7%	0.8%	1.0%	2.3%	2.1%	2.2%
Baker River	74	**81.2%**	0.9%	1.8%	5.6%	2.6%	4.1%	3.8%
Cascade River	98	0.3%	**98.2%**	0.2%	0.3%	0.3%	0.3%	0.3%
Big Creek	96	0.5%	0.4%	**97.7%**	0.4%	0.4%	0.3%	0.3%
Clear Creek	98	3.2%	0.6%	0.6%	**93.5%**	1.0%	0.6%	0.5%
Finney Creek	85	1.4%	0.3%	0.5%	0.5%	**95.8%**	0.9%	0.6%
Ross Lake '06	48	2.2%	0.4%	1.2%	0.9%	1.2%	**90.0%**	4.1%
Ross Lake '09	40	0.6%	0.5%	0.4%	0.5%	0.5%	**97.2%**	0.5%
Ross Lake '10	47	0.6%	0.2%	0.3%	0.3%	0.5%	**97.6%**	0.5%
Dry Creek	62	0.8%	0.5%	0.4%	0.5%	0.6%	**96.4%**	0.8%
Roland Creek	97	0.7%	0.4%	0.4%	0.5%	0.7%	**96.6%**	0.6%
Diablo Lake	62	4.5%	1.1%	2.8%	1.9%	1.5%	**87.0%**	1.2%
Stetattle Creek	76	8.6%	0.8%	2.6%	2.5%	1.8%	**82.3%**	1.4%
Blackwater River	67	0.8%	0.6%	0.5%	0.5%	0.7%	15.3%	**81.6%**

Table 22b. Proportion of genetic ancestry to one of three groups for collections of rainbow trout from the upper Skagit River.

	N =	1	2	3
Stetattle Creek	76	**71.2%**	18.4%	10.4%
Diablo Lake	62	**82.3%**	10.2%	7.6%
Ross Lake '06	48	**58.1%**	**35.3%**	6.6%
Dry Creek	62	12.1%	**74.6%**	13.3%
Roland Creek	97	14.1%	**71.0%**	14.8%
Ross Lake '09	40	16.4%	**45.5%**	**38.1%**
Ross Lake '10	47	8.4%	19.6%	**72.0%**

Analysis of the natural-origin adult and juvenile steelhead from the same watersheds revealed no significant differences with the tests of genotypic differentiation and pairwise F_{ST} values that were not significantly different from zero with exception of the adults and juveniles from the Sauk River. Comparison of the temporal collections of juveniles revealed significant differences between the Finney Creek collections in 2009 and 2010 and the collections from the Cascade River that were taken in 2009 and 2010. All comparisons of resident rainbow trout to the adult and juvenile steelhead collections from the same watershed were significantly different.

Analysis of the five of seven temporal collections from the Sauk River adults, spanning 30 years, revealed no significant differences among any of the collections (Table 23). Two collections were not used due to small (10 or less) sample size.

Table 23. Pairwise F_{ST} values (above diagonal) and genotypic differentiation (below diagonal) for five collections of steelhead collected from the Sauk River. Values in bold identify pairwise comparisons that are not significantly different from zero and genotypic differentiation values that were not significantly different.

	Sauk R. '81	Sauk R. '83	Sauk R. '09	Sauk R. '10	Sauk R. '11
Sauk R. '81	****	**0.0017**	**0.0051**	**-0.0005**	**0.0044**
Sauk R. '83	**0.5751**	****	**0.0022**	**-0.0017**	**0.0012**
Sauk R. '09	**0.0718**	**0.2152**	****	**-0.0028**	**0.0011**
Sauk R. '10	**0.8553**	**0.4236**	**0.8251**	****	**0.0002**
Sauk R. '11	**0.0849**	**0.5837**	**0.6787**	**0.6822**	****

8.3.5 Stock of Origin Analysis of Fisheries

The 100 percent simulations run in the realistic fishery simulation model by Anderson et al. (2008) was conducted and identified a probability of assignment to Skagit natural-origin steelhead (an aggregate of the natural-origin Skagit River basin collections), Marblemount Hatchery, and Chilliwack Hatchery (Table 24). Assignment back to population of origin was over 95 percent for all three collections (95 percent confidence interval was between 92 – 100 percent).

Table 24. Results of the 100 percent simulations using the model by Anderson et al. (2008) for natural- and hatchery-origin populations of steelhead in the Skagit River basin. Point estimates and the 95% confidence intervals are shown for each of three aggregate groups.

		95% Confidence	
Population	Estimate	Lo	High
Skagit River natural-origin	0.9977	0.9867	1.0000
Marblemount Hatchery	0.9975	0.9846	1.0000
Chilliwack Hatchery	0.9580	0.9224	0.9918

The leave one out jackknife analysis on the same three groups included in the 100% simulation analysis resulted in over 93% correct assignment for both the Skagit River natural-origin and Marblemount Hatchery collections and 86.5% correct assignment for the Chilliwack Hatchery collection (Table 25). These test results indicated that if fish from these three groups occurred in a sample in which origin was unknown, such as in a mixed harvest group, each fish could be assigned with high accuracy to their actual population (stock) of origin.

Table 25. Jacknife of the three steelhead baseline aggregate collections. Rows identify the number (a) and percentage (b) of individuals that assign back to each baseline stock. Columns identify the total percentage of individuals that assign to a baseline stock from each collection. Shaded boxes indicate assignments back to the original stock of origin.

a - number of individuals

	natural-origin	Marblemount Hatchery	Chilliwack Hatchery
natural-origin	249	12	6
Marblemount Hatchery	8	124	0
Chilliwack Hatchery	5	0	32

b - percentage of individuals

	natural-origin	Marblemount Hatchery	Chilliwack Hatchery
natural-origin	93.3%	4.5%	2.2%
Marblemount Hatchery	6.1%	93.9%	0.0%
Chilliwack Hatchery	13.5%	0.0%	86.5%

Hatchery and wild stock composition of fishery samples was influenced by the type of fish sampled during each fishery (Table 26). The samples that were collected during the 1994 and 2010 fisheries included an unknown mixture of both unclipped and adipose clipped steelhead while the samples from the 2009 fishery were all defined as unclipped. In 2008, one collection (08MR) included the unclipped steelhead and another (08MU) included adipose clipped steelhead. The samples from 1994 and 2010 had over 84% of the individuals assign as natural-origin. The samples from 2008 identified as unclipped were nearly all assigned to the Skagit natural-origin collection while the 2009 samples identified as unclipped had only 70% assignment to the natural-origin collection. The one collection from 2008 that were identified as hatchery-origin (clipped) only included 10 samples and 77% assigned as hatchery-origin.

Table 26. Mixture proportions of five Skagit River basin steelhead fishery samples as natural-origin or hatchery-origin (Marblemount or Chilliwack) using the program ONCOR. Samples from the fisheries were identified as unclipped or clipped at the time they were collected as identified in the footnote.

	94AAA	08MR	08MU	09JC	10AE
	N = 42	N = 124	N = 10	N = 97	N = 72
natural - origin	0.8772	0.9779	0.2349	0.7004	0.8457
Marblemount Hatchery	0.1228	0.0000	0.7651	0.2996	0.1059
Chilliwack Hatchery	0.0000	0.0221	0.0000	0.0000	0.0484

WDFW code	Fishery collections
94AAA	Skagit River fishery 1994 - unclipped and clipped adults
08MR	Skagit River fishery 2008 - unclipped adults
08MU	Skagit River fishery 2008 - clipped adults
09JC	mid Skagit River Tribal Fishery 2009 - unclipped adults
10AE	mid Skagit River Tribal Fishery 2010 - unclipped and clipped adults

8.4 Discussion/Conclusions

Anadromous adult steelhead, juvenile *O.mykiss* (collected in anadromous zones), and resident rainbow trout (collected above impassible barriers) were collected from the Skagit River to establish a baseline for comparisons throughout the basin. Each mainstem reach and primary tributary was assumed to potentially represent a different genetic stock; therefore samples were collected from as many of these areas as possible. Samples from Marblemount Hatchery were also included to determine if natural-origin steelhead were differentiated from hatchery-origin. All samples were thought to represent the winter run of steelhead with the exception of some early returning fish to Marblemount Hatchery and some steelhead that were smaller than the average returning fish (defined as ¼ pounders) captured in the middle Skagit River. There was some question if some of the earlier returning steelhead could be from the Chilliwack Hatchery in British Columbia; therefore we included a collection from the broodstock for comparison.

The 30 collections of adults from individual tributaries and the hatcheries were combined into nine aggregate groups because the temporal collections were not differentiated. Analysis of the genotypic differences among all of the natural-origin adult steelhead showed a lack of differentiation among steelhead in mainstem reaches and tributaries. The lack of differentiation suggests there has been mixing of steelhead throughout the basin or that the Skagit River basin had one ancestral steelhead that has occupied each of the mainstem reaches and tributaries. The natural-origin steelhead were significantly differentiated from both the Marblemount and Chilliwack Hatcheries. The factorial correspondence plot identified more separation of the natural-origin steelhead with the Marblemount hatchery than the Chilliwack Hatchery. The source of broodstock for the Marblemount Hatchery is from Chambers Creek in south Puget Sound so not too surprising to be differentiated. Kassler et al. (2010) found significant differences in the natural-origin steelhead in the Hoh River compared to the out of basin hatchery steelhead. The Chilliwack Hatchery uses natural-origin steelhead from the Chilliwack River for their broodstock each year; therefore we anticipated these samples to be different from both the natural- and hatchery-origin steelhead in the Skagit River basin.

WDFW developed a methodology to calculate introgression between individuals of natural- and hatchery-origin (see section 10). We can evaluate collections as a whole using the allele frequencies of all individuals in a collection to calculate if a significant difference exists. However, analysis of individual steelhead to determine if they have genetic contribution that is from natural and hatchery-origin needs to be addressed. Natural-origin steelhead from the Skagit River and hatchery-origin steelhead at Marblemount Hatchery (Chambers Creek, Puget Sound origin) likely have a recent common ancestor therefore they will share a portion of their genetic profile. Because they share some amount of genetic information we are not able to say that a common allele between individuals is the result of successful spawning together versus a shared allele.

8.4.1 Genetics of Natural-Origin Juvenile and Adult Steelhead

The genetic difference among the collections of juvenile O.mykiss from anadromous zones was varied. Some of the temporal collections from the same location were significantly different while not significantly different from the collections of adults from other tributaries.

The population statistics to evaluate the possibility of small sample size, family groups, or mixture from multiple sources revealed a few collections that suggest we may not have had random samples from those locations resulting in the significant values that were calculated. Overall, the juvenile collections were not differentiated from the adult collections in the same watersheds with the exception of the adult and juvenile collections from the Sauk River. Originally we had defined an upper and lower reach of the Sauk River; however we were unable to obtain enough samples collected in the upper Sauk and samples were combined into one aggregate collection group. The difference between the adult and juvenile collections may be a representation of small differences within the upper and lower reaches of the Sauk River that were not present in both adult and juvenile collections.

8.4.2 Genetics of Natural-Origin Juveniles Outside Anadromous Zone

Collections of rainbow trout taken above impassible barriers were significantly different. Each of these collections was different to all other collections suggesting isolation from any of the anadromous steelhead. The exception was with the Baker River collection of resident rainbow trout that were not significantly different from the Skagit River adult steelhead. Historically, anadromous steelhead adults were trapped from the mouth of the Baker River and hauled and released in Baker Lake to spawn; therefore samples that were collected could be the offspring of anadromous steelhead that have residualized in Baker Lake. Juvenile Baker samples were collected from the downstream collection facility suggesting that these were smolts expressing anadromy.

The above reservoir upper Skagit River resident rainbow trout were analyzed with collections of rainbow trout and with the adult steelhead in the Skagit River basin to determine if they were differentiated or genetically similar. The analysis of the above reservoir upper Skagit River basin rainbow trout revealed significant differences among all of the collections from the above reservoir upper Skagit River with exception of Dry Creek and Roland Creek. Dry and Roland Creeks are both tributaries to Ross Lake; however they are both differentiated from the Ross Lake fishery collection suggesting that there are several genetic sources of rainbow trout found in Ross Lake or they have been introduced. A portion of the rainbow trout in Ross Lake spawn in the section of the Skagit River that is in Canada (per. comm., Ed Connor, 2012) and possibly represent another genetic source. The three collections from Ross Reservoir (2006, 2009, and 2010) are also all differentiated from each other suggesting multiple sources or differences among the year classes of rainbow trout in Ross Reservoir. The other above reservoir upper Skagit River rainbow trout collections are also differentiated from the other collections suggesting little migration among the upper Skagit reservoirs resulting in the populations remaining differentiated. Gorge Reservoir was not included in the analysis, but may share a genetic similarity to the collection from Stetattle Creek given that it is a direct tributary. The collections of rainbow trout in the above reservoir upper Skagit River represented a genetically differentiated group of *O.mykiss* as was seen with the other collections of rainbow trout. They were also significantly differentiated from the collections of adult steelhead collected throughout the Skagit River basin.

Four different tributaries had large enough sample sizes of adult, juvenile, and resident rainbow trout to compare. There was some speculation that resident rainbow trout found above the barriers move downstream into the pools below the barriers and the juvenile *O.mykiss* collected would include some genetic similarity to the resident rainbow trout. The analyses provide evidence that the adult and juveniles are all similar and that the resident rainbow trout are not genetically similar to any of the collections of anadromous fish. This suggests the lack of forced or behavioral movement from above the barrier into the lower reaches of the river. A limiting factor may be the survival of the individuals that are going downstream.

8.4.3 Genetic Profile of Natural-Origin Sauk Adult Steelhead

Seven temporal samples of adult steelhead were collected from the Sauk River spanning 30 years. Collections were taken from scales provided by hook and line recreation fishing in the 1980's and then through the efforts of this project from 2008 – 2011. The goal of analyzing steelhead samples over a 30 year time period was to evaluate the genetic profile of steelhead. We were assessing if there had been any changes over time through reduction in population size or from hatchery-origin steelhead reproducing successfully in the Sauk subbasin. In either scenario we would anticipate a genetic change between the samples collected in the 1980's to the most recent samples; however genotypic tests identified that there were no significant differences and pairwise analyses of F_{ST} identified all comparisons were not significantly different from zero. These tests provide data to show that the genetic profile of steelhead within the Sauk River has not changed over the 30 year time period.

8.4.4 Genetic Distinction Level Between Adult Collection Groups

The fishery samples were analyzed using a baseline that was established by aggregating the natural-origin collections, Marblemount Hatchery collections, and the collection from Chilliwack Hatchery into three separate groups. This aggregated baseline was evaluated with the 100% simulation test and jackknife analysis to show that the three groups were genetically distinct and to show how well unknowns would assign back to the correct stock. The 100% simulation and jackknife analysis both identified that the aggregate groups were genetically distinct and that and an unknown sample could be correctly assigned to the stock of origin.

The fishery samples were analyzed with a baseline that included natural-origin steelhead and hatchery-origin steelhead from Marblemount and Chilliwack Hatcheries. This analysis of the fishery samples was conducted to determine the extent of the impacts that fisheries were having on the targeted portion of the steelhead run. In the collections from 1994 and 2010 there were both unclipped and clipped steelhead and the mixture proportions indicated greater than 84% of the individuals as natural-origin. It is unknown how many of the individual samples were clipped; therefore the high percentage of individuals that were assigned as natural-origin may be misleading. The other fisheries samples collected in 2008 and 2009 were supposed to be unclipped steelhead; however in the 2009 collection only 70% of the mixture proportion was assigned to the natural-origin collection. The collection in 2008 included clipped samples only, but greater than 23% of the mixture assigned to the natural-origin. The scale patterns for each of the samples needs to be analyzed to determine if some of the samples collected as unclipped were of hatchery-origin and therefore misidentified by external marks. Some of the assignments as hatchery-origin could also be an indication that natural- and hatchery-origin individuals have spawned together.

The homogeneous genetic makeup of the natural-origin steelhead in the Skagit suggests that there has been significant mixing within the population. Reduced spawning location fidelity is considered to be a logical explanation for this outcome. There appears to be a large enough proportion of the population that does not return to their natal spawning area such that over many generations the genetic makeup of the population has become blended. In contrast to this apparent reduction in spawning area fidelity within the Skagit basin is what was recently revealed about Skagit bull trout populations. Smith (2010) quantified the level of genetic diversity within and among bull trout populations in subbasins of the Skagit River. Unlike steelhead, Smith found that bull trout collections from several mainstem Skagit reaches and numerous tributaries all showed strong evidence for genetic differentiation and low gene flow among reporting groups. All groups remained genetically segregated likely the result of high natal spawning area fidelity. Genetic segregation within the Skagit watershed is being exhibited by bull trout while steelhead, another anadromous species appears to have followed a different strategy of reduced spawning location fidelity (straying).

9.0 Hybrid Density in Juvenile and Adult Steelhead on a Spatial Level

9.1 Introduction

The presence and density of hybrid juveniles and adult steelhead in the Skagit that result from reproductive cross-breeding between hatchery and natural-origin adults outside of the hatchery could have many genetic and ecological consequences capable of compromising the productivity and genetic integrity of natural-origin steelhead populations. Several recent studies have shown that hatchery-origin steelhead and hybrids have lowered reproductive success than their natural-origin counterparts due to a lack of local adaption and from the effects of domestication (Kostow et al. 2003; Araki et al. 2007a; Araki et al. 2008; Chilcote et al. 2011; Fraser et al. 2011).

Kostow (2009) found that there are many ecological risks associated hatchery steelhead spawning outside of the hatchery. Some of the most important contributing factors were identified as; relative abundance of hatchery and natural-origin fish in natural production areas, hatchery programs that increase density-dependent mortality, residual hatchery-origin fish, and some physical advantages that hatchery fish can have over wild fish. Any one of these factors is capable of compromising the productivity and genetic integrity of natural-origin steelhead populations.

An assessment of putative hybrids found in the Skagit watershed was used to establish the presence and relative densities of hybrids from a spatial standpoint. The nature and extent of hybridization measured within the natural-origin population at the juvenile and adult stages can be used to form a better understanding of the inherent genetic and ecological risk to the indigenous population.

9.2 Methods

The ancestry for steelhead individuals from the Skagit River basin were evaluated using a Bayesian analysis implemented in the program STRUCTURE 2.1 (Pritchard et al. 2000). Structure uses an iterative process to partition the dataset into a number of genetic clusters (populations) which minimizes Hardy-Weinberg disequilibrium and linkage disequilibrium in the clusters. This model-based method can be used to infer population structure and assign individuals to populations. Individual adult and juvenile steelhead from spatially separated regions within the Skagit were probabilistically assigned to a population or jointly to both populations (natural-origin or Marblemount hatchery) if their genotypes indicate that they are admixed, a mixture from two or more distinct genetic populations (potential hatchery-wild hybrids). The Pritchard et al. (2000) model was developed to demonstrate the presence of population structure, study hybrid zones and identifying admixed individuals. The results derived from STRUCTURE were used to understand the relative density of hybridization on a spatial level based on the identification of admixed individuals present in collections from within the Skagit watershed. The juveniles used in this evaluation were from young-of -year size class and thereby much smaller in length than smolt released from the Marblemount hatchery.

The ancestries of individual young-of-the year juvenile and adult steelhead were evaluated using STRUCTURE 2.1 (Pritchard 2000). Admixed individuals from these collections were indentified as natural-origin/Marblemount hatchery (Chambers) hybrids if Chambers ancestry exceeded 20% and did not exceed 90%. Individuals containing more than 90% Chambers ancestry were further indentified as resulting from naturally spawned parents; hatchery adults spawning naturally outside the hatchery (Natural Spawn Hatchery). Results for the Cascade River adult collection are absent because adequate sample numbers were not obtained. STRUCTURE ancestry results for individual fish from each collection area are presented in Appendix 2.

9.3 Results

9.3.1 Natural-Origin Juveniles

The juvenile collections showed the presence of presumptive hybrids in all collection areas sampled (Figure 21 and Table 27). The hybrid densities from the Skagit collection areas ranged from 6% in the Sauk collection area to 32.7% in Finney Creek. The two collection areas with the highest hybrid percentages were both from middle Skagit River tributaries; Finney and Grandy creeks.

The presence of young-of-the-year juvenile resulting from naturally spawned hatchery parents occurred in six of the eight collection locations. Bacon and Diobsud creeks were the only two locations devoid of juveniles resulting from two hatchery parents. Juvenile densities from the other four collection locations ranged from 1.1-10.6%. The two collection areas with the highest incidence were Finney and Grandy creeks, both middle Skagit tributaries.

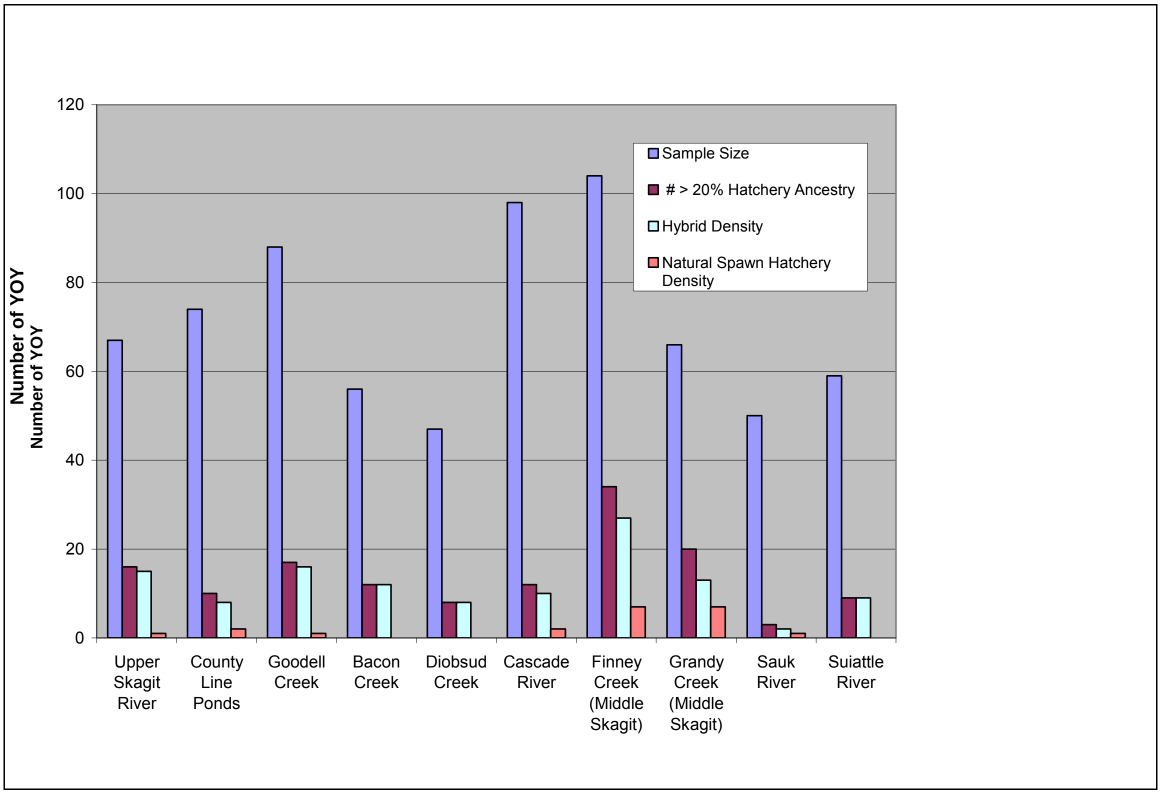


Figure 21 - Comparison of Hybrid Density from YOY Juvenile Steelhead Collections

Table 27. Spatial Comparison of Hybrid Density from YOY Natural-Origin Juvenile Steelhead Collections

Juvenile Steelhead							
Location	Sample Size	# > 20% Hatchery Ancestry	% > 20% Hatchery Ancestry	Hybrid Density	Hybrid Density (%)	Natural Spawn Hatchery Density	% Natural Spawn Hatchery
Upper Skagit River	67	16	23.9%	15	22.4%	1	1.5%
County Line Ponds	74	10	13.5%	8	10.8%	2	2.7%
Goodell Creek	88	17	19.3%	16	18.2%	1	1.1%
Bacon Creek	56	12	21.4%	12	21.4%	0	0.0%
Diobsud Creek	47	8	17.0%	8	17.0%	0	0.0%
Cascade River	98	12	12.2%	10	10.2%	2	2.0%
Finney Creek (Middle Skagit)	104	34	32.7%	27	26.0%	7	6.7%
Grandy Creek (Middle Skagit)	66	20	30.3%	13	19.7%	7	10.6%
Sauk River	50	3	6.0%	2	4.0%	1	2.0%
Suiattle River	59	9	15.3%	9	15.3%	0	0.0%

9.3.2 Natural-Origin Adults

The adult collections also show the presence of presumptive hybrids in all collection areas sampled (Figure 22 and Table 28). The hybrid densities within the Skagit collection areas ranged from 15.4% in the middle Skagit collection area to 35.8% in Finney Creek. In contrast, of the 169 adult hatchery steelhead from the Marblemount hatchery collection, only a single fish was identified as a putative hybrid. In addition, 3 of the 5 Skagit collection-group areas showed some incidence of natural spawned hatchery adults. The upper Skagit, Sauk and Finney collection areas exhibit low levels of naturally spawning hatchery adults mating together ranging from 1.9-4.9% (Table 28). The middle Skagit and Suiattle rivers on the other hand showed no evidence of hatchery fish naturally spawning with each other at the adult level.

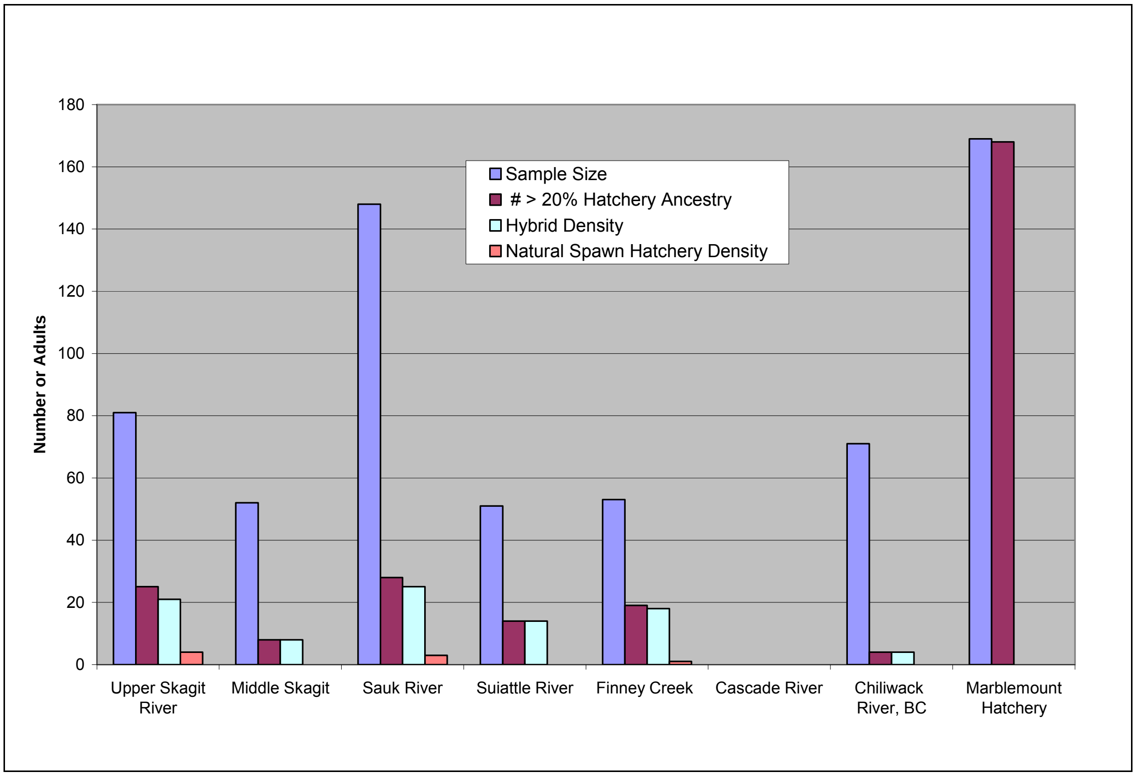


Figure 22 . Comparison of Hybrid Density from Adult Steelhead Collections

Table 28. Spatial Comparison of Hybrid Density from Adult Steelhead Collections based on genetic ancestry data

Adult Steelhead							
Location	Sample Size	# > 20% Hatchery Ancestry	% > 20% Hatchery Ancestry	Hybrid Density	% Hybrid	Natural Spawn Hatchery Density	% Natural Spawn Hatchery
Upper Skagit River	81	25	30.9%	21	25.9%	4	4.9%
Middle Skagit	52	8	15.4%	8	15.4%	0	0.0%
Sauk River	148	28	18.9%	25	16.9%	3	2.0%
Suiattle River	51	14	27.5%	14	27.5%	0	0.0%
Finney Creek	53	19	35.8%	18	34.0%	1	1.9%
Cascade River	nd	-	-	-	-	-	-
Chiliwack River, BC	71	4	5.6%	4	5.6%	0	0.0%
Marblemount Hatchery	169	168	99.4%	1	0.6%	-	-

9.3.3 Hatchery Adults

When the same ancestry evaluation is applied to hatchery-origin steelhead from the Marblemount facility the data shows that only 0.6% of the hatchery-origin adult population contained greater than 20% natural-origin ancestry (Table 28). The hybrid density in the hatchery population is quite low compared with hybrid densities levels in natural-origin adults and juveniles.

9.4 Discussion/Conclusions

There are multiple ecological and genetic implications associated with the presence of hatchery-origin steelhead juveniles in the natural production zone and adults spawning naturally outside of the hatchery. The many ecological and genetic risks associated hatchery steelhead spawning outside of the hatchery were recently described by Kostow (2009) and Seamons (2010). Kostow identified a number of factors that singularly or in combination are capable of compromising the productivity and genetic integrity of natural-origin steelhead populations. She found that ecological risks occur when the presence of hatchery fish affects how natural-origin fish interact with their environment or with other species. Some of the most important contributing factors were identified as; relative abundance of hatchery and natural-origin fish in the natural production areas, hatchery programs that increase density-dependent mortality, residual hatchery-origin fish, and some physical advantages that hatchery fish can have over wild fish.

Seamons studied the strategy of segregation by divergent lifehistory in steelhead where hatchery-origin fish were selected to spawn months earlier than the indigenous wild population. This is the same strategy that has been used for 60-years in the Skagit watershed with steelhead. After three generations, Seamons found that the genetic makeup of the indigenous population had shifted with the proportion of natural-origin ancestry smolts and adults declining by 10-20%. In addition, he found that up to 80% of the naturally produced steelhead were hatchery-natural-origin hybrids. Finally, he discovered that the hybrid proportions in both smolts and adults shifted quickly relative to the number of naturally spawning hatchery fish present.

Numerous researchers have found that non-native broodstock like that used at the Marblemount hatchery often have lower reproductive success than their natural-origin counterparts due to a lack of local adaption and from the effects of domestication (Kostow et al. 2003; Araki et al. 2007a; Araki et al. 2008; Chilcote et al. 2011; Fraser et al. 2011). Reproductive success of hatchery steelhead from the Marblemount facility has shown a consistent downward trend over the past two decades (see section 11). Because of this reduction in reproductive success interbreeding between hatchery and natural-origin steelhead in the Skagit basin may contribute to long term negative fitness consequences.

The initial stage of genetic introgression thought to take place in the Skagit results from hatchery and natural-origin steelhead reproducing together. These are considered an F1 hybrid because the progeny result from two distinctly different parent types. If the F1 progeny survive to adulthood another level of introgression can occur that involves three other predictable mating combinations. These include the following mating types; F1 progeny x F1 progeny, F1 progeny x hatchery-origin adult and F1 progeny x natural-origin adult. Assuming some survival to adulthood results from each of these mating types, over multiple generations it is reasonable to assume that the population would be comprised of some individuals displaying the full range of possible hatchery/natural-origin genetic ancestry. From each of the collection areas studied there were examples of individuals from each location displaying the full range hybrid ancestry at both the juvenile and adult level (see appendix 2). The amount of hatchery ancestry for individual fish ranged from 20% to over 90% in nearly all areas (collections). Distance from the hatchery did not appear to influence the hybrid density observed in juveniles or adults collections. Based on our data we speculate that hatchery or hybrid adults stray and spawn throughout the watershed. Because both natural and hatchery-origin steelhead found in the Skagit watershed appear to have shown the tendency to stray within the basin it seems possible that both hatchery-origin and hybrid adults may both be transferring hatchery ancestry throughout the basin. Schroeder (2001) found that hatchery steelhead straying behavior was ubiquitous in the coastal Oregon rivers he studied. He concluded that the widespread occurrence of straying by hatchery fish presented both genetic and ecological risks to wild populations of winter steelhead.

We also found evidence of straying hatchery adults naturally spawning together throughout the basin. In general, the incidence of individual fish with hatchery ancestry in excess of 90% was slightly higher at the juvenile level than for adults. Six of the 8 juvenile collection areas showed varied levels of hatchery x hatchery parentage as compared with 3 of the 5 adult collection areas. It appears that hatchery-origin fish are spawning successfully with each other outside the hatchery and progeny are surviving at both the juvenile and adult level.

The long-term manifestation of physical segregation is displayed by comparison of the ancestry data from the Marblemount hatchery and natural-origin populations. Ancestry data from the Marblemount (Chambers stock) adults demonstrates that genetic input from the natural-origin population appears to be limited as would be expected with a segregated hatchery program where natural-origin adults can be physically excluded from entering the hatchery. From the hatchery standpoint physical segregation appears to be producing the desired outcome resulting in only 0.6% of hatchery adults exhibiting levels of natural-origin ancestry (>20%) that would be expected from hybridization with natural-origin fish. The remaining 99.4% of the hatchery adults examined contained lower levels of natural-origin ancestry (<20%). When the natural-origin threshold is lowered from <20% to <5%, seventy-three percent of the hatchery adults continue to meet this more stringent standard. After more than 60-years of Chambers based hatchery production on the Skagit the infusion of natural-origin ancestry remains extremely low in the hatchery stock. Good hatchery practices exercised during this period appear to have consistently prevented natural-origin adults from entering the hatchery facility.

While it appears that natural-origin adults are physically prevented from reproducing with hatchery-origin adults inside the hatchery facility, our ancestry data suggests that a weakness of the segregated program used on the Skagit is that hatchery fish can not be physically, temporally or behaviorally prevented from straying and spawning naturally with other hatchery strays or natural-origin adults. Although this core principle of segregated programs is critical to protect the genetic integrity of the natural-origin steelhead it does not appear to be working as effectively as desired on the Skagit or in some other systems studied (Kostow 2009, Seamons 2010). The observed Skagit outcome is somewhat predictable when the contributing factors are considered collectively.

First, hatchery managers have no effective tool to prevent hatchery-origin steelhead from choosing to spawn outside of the hatchery. Secondly, we have shown that both hatchery and natural-origin adults exhibit a level of straying. For hatchery strays this leads to reproduction alternatives outside the hatchery that include other hatchery strays, natural-origin or hybrid adults that are all found throughout the basin in varied densities. The final factor involves temporal separation of spawn timing between hatchery and natural-origin populations. The data presented from this study provide evidence showing that there are overlaps in spawn timing that exist in the Skagit watershed. The spawn time overlap may vary by return year of hatchery-origin adults and the presence of hybrids as described by Seamons.

Little is known about whether there is a genetic component to return timing in salmonids. However, what little research is available suggests that for steelhead, once hybridization occurs the temporal separation of spawn timing for these individuals may be compromised. Seamons suggests that the migration and spawn timing of hybrid steelhead would be expected to overlap with the timing of both populations. His results showed that putative steelhead hybrids tended to spawn later than expected and closer to the spawning season of the indigenous population. This same research and ours found that late returning non-hybrid hatchery-origin adults, especially males, on the Skagit were found to stay in fresh water for many months. Other studies found that hatchery males in particular are capable of remaining in fresh water until natural-origin females arrive and mate with wild fish throughout the wild spawning season, thus producing offspring with relatively late return timing (Leider et al. 1984; Seamons et al. 2004). On the Skagit it appears that the largest overlap in spawn timing occurs in the middle Skagit reach, especially in the tributaries where some of the earliest natural-origin spawning takes place.

It remains unclear how the hybrid densities within the Skagit change over the course of multiple reproductive generations. Although we suggest that there are many factors in force influencing the density of putative hybrids in the natural population, this study presents a set of genetic data from Sauk River adults that spans 30-years (see section 8). This dataset, while only providing a cursory assessment, suggests that the scope of Skagit hybridization has remained fairly static over this three decade period.

If fish managers decide to reduce the observed levels of hybridization present in the Skagit basin it logically stem from a reduction in the size of the hatchery release since segregation by life history differences (spawn time) have failed to prevent undesired genetic and ecological interactions.

10.0 Introgressive Hybridization

*Special note: this chapter was written by Ken Warheit (WDFW). The narrative is written in the first person.

10.1 Introduction

Fish and wildlife managers have been translocating and artificially propagating animals for decades, generally for the purpose of conserving declining or depressed populations or species, or for supplementing a population that is exploited for commercial or recreational harvest (Frankham et al. 2002, Naish et al. 2008, Laikre et al. 2010). The use of hatcheries to enhance salmon and steelhead populations has been extensive, and our understanding of their effects on wild populations is growing (e.g., Waples 1991, Hilborn 1992, Araki et al. 2007, Laikre et al. 2010, Thériault et al. 2011, Christie et al. 2012, Hess et al. 2012, Seamons et al. 2012, Zhivotovsky et al. 2012). These effects can be categorized as either ecological (e.g., competition), or genetic (e.g., domestication, hybridization) (Naish et al. 2008, Kostow 2009). To moderate or eliminate the negative effects to wild populations the Hatchery Scientific Review Group (HSRG) recommended that every salmonid hatchery develop a genetic management plan, and every hatchery population be managed as either segregated from or integrated with the wild population(s) that spawn naturally within the basin (HSRG 2004, Mobrand et al. 2005). The intent of segregated hatchery programs is to keep separate the hatchery and wild populations, and they are managed so that only hatchery-origin individuals are used as broodstock, and hatchery-origin adults are restricted from spawning naturally, with the understanding that any natural spawning by hatchery-origin fish from the segregated program will impose potentially unacceptable risks to natural populations. Therefore, by design the hatchery and wild populations in segregated programs are genetically distinct, and the degree of genetic differentiation is a function of the source of the hatchery broodstock, hatchery founder effect, genetic drift, or domestication selection (Mobrand et al. 2005). Primarily, the purpose of segregated hatchery programs is to create harvest opportunities, and secondarily, to direct harvest away from wild populations of conservation concern (e.g., mark-selective fishery). However, because segregated hatchery programs assume that hatchery and wild populations will remain distinct, an unintended consequence of a segregated hatchery program is hybridization between hatchery-origin and wild fish that spawn naturally. Hybridization may be unavoidable if fishery managers lack the ability to restrict hatchery-origin fish from natural spawning grounds, and if spawning by hatchery-origin and wild fish is not segregated spatially or temporally (Waples 1991, Naish et al. 2008).

Hybridization that is anthropogenic in origin has caused the extinction or decline of animal taxa either by the replacement of natural taxa by their hybrids, loss of fitness or adaptation through genetic mixing, loss of genetic diversity or change in population structure, and reduction in effective population size (Ryman and Laikre 1991, Waples 1991, Allendorf et al. 2001, Laikre et al. 2010, Christie et al. 2012). Despite fishery managers' intent to keep hatchery and wild populations segregated, introgressive hybridization occurs, and may occur at a high rate (e.g., Seamons et al. 2012).

For the better part of half a century, hatchery production of steelhead trout (*Oncorhynchus mykiss*) in western Washington State has mainly propagated and outplanted two populations: winter-run from Chambers Creek and summer-run from the Washougal River (i.e., Skamania Hatchery) (Crawford 1979). The Chambers Creek hatchery stock was derived originally in 1945 from a now-extinct run in Chambers Creek, Tacoma, Washington, and cultivated at South Tacoma Hatchery (now Lakewood Hatchery). This broodstock was cultivated and used in segregated winter-run hatchery programs throughout Puget Sound, Washington outer coast, and lower Columbia River. Thus, these hatchery programs have been spawning and releasing out-of-basin fish into streams that are home to indigenous natural populations since the mid-1950s. Recreational and tribal harvest of winter steelhead in the Skagit River basin has been supported by out-of-basin hatchery fish planted into the system since 1950 (Washington Department of Fish and Wildlife 2012). To adequately maintain these fisheries, since the 1960s, Marblemount Hatchery (Cascade River/Clark Creek, approximately RM 78 of the Skagit River) has propagated a Chambers Creek-lineage winter steelhead population. As in all Chambers Creek-based hatchery programs, the Marblemount Hatchery winter steelhead program is managed as a segregated population by its use of only hatchery-origin broodstock. However, there is no effective mechanism to remove from the river those hatchery-origin steelhead that do not return to the collection facility at Marblemount Hatchery; therefore, hatchery-origin fish are free to spawn naturally with each other and with wild steelhead when their occurrences overlap spatially and temporally.

The intent of this study is to determine the extent to which the segregated Marblemount Hatchery winter steelhead population has hybridized with wild steelhead populations(s) within the Skagit River Basin. Since all native winter steelhead populations in Puget Sound (including Chambers Creek fish) are closely related to each other (Ostberg and Thorgaard 1999, Busby et al. 1996, Hard et al. 2007) the Chambers Creek derived Marblemount Hatchery population shares a relatively recent common ancestor with the wild populations within the Skagit River.

Since a hybrid individual will show genetic similarities to both parent populations, to adequately document hybridization between two closely related populations, one would need to differentiate similarity due to recency of common ancestry from similarity due to introgressive hybridization[1].

This report combines elements of two projects, both of which aimed to quantify hatchery-wild introgressive hybridization. In the first project, I used simulated individuals from two populations to determine the power in our dataset to detect their hybrids, given a set of differentiations between the two populations (measured by F_{ST}) and number of loci scored. In the second project, I evaluated introgressive hybridization between the segregated population from Marblemount Hatchery and the wild and native steelhead populations within the Skagit River Basin. This second project had two components: (1) an empirical model, used in conjunction with the simulations described briefly above, to define the statistical limits of the analysis, and (2) a quantitative assessment to determine the relative degree of introgressive hybridization between hatchery and wild populations for 10 juvenile and five adult collections

[1] Introgressive hybridization is defined as hybridization that results in the establishment of exogenous alleles within a population; here, the presence of hatchery alleles within a wild population. I differentiate introgression (state) from gene flow (process). That is, gene flow is the process that gives rise to the state of introgression.

from the Skagit Basin. From these analyses, I concluded that although introgressive hybridization between hatchery and wild Skagit River populations can be detected, there is insufficient power with currently available data to quantify the degree to which these populations are introgressed. However, it appeared that certain wild Skagit River populations have a stronger and more consistent signal of introgression than other populations.

10.2 Methods

A description of the 15 Stevan Phelps Allele Nomenclature (SPAN) microsatellite loci, population genetic parameters, and methods to calculate these parameters was presented by Kassler and Warheit (2012). I limited the analysis here to a subset of the samples from Kassler and Warheit (2012) choosing only individuals with complete genotypes, and juvenile and adult collection aggregates (henceforth, populations) with original samples sizes greater than four (Figure 23, Table 29; see also Kassler and Warheit 2012).

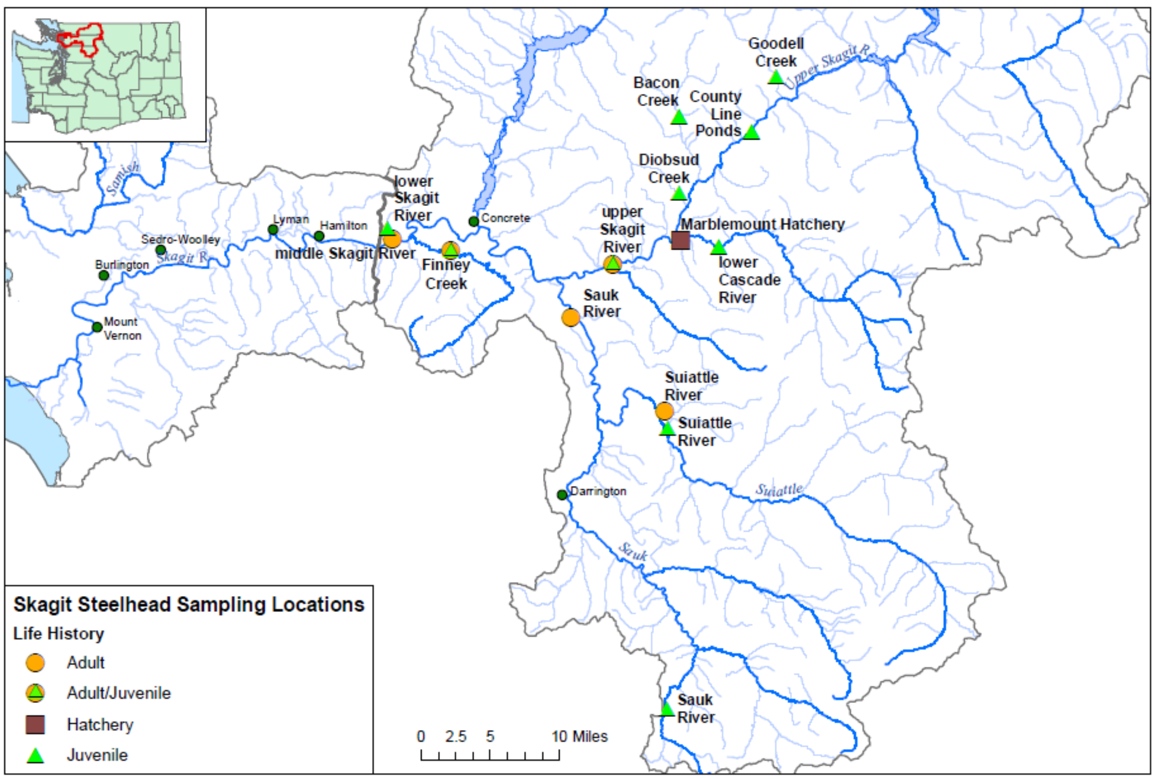


Figure 23. Skagit River and geographic distribution of sampling locations (populations). Grandy Creek here is labeled as lower Skagit River.

Table 29. Origin, collection codes, and geographic distribution of samples used for the analyses in this report. These samples are a subset of those described in Kassler and Warheit (2012), limited here to individuals with complete genotypes (i.e., no data missing from any of the 15 microsatellites). A subset of the wild adult samples was used as parents (Par) and for the analysis (Analy) in the empirical model (see text). The numbers in each collection code denote the year the samples were collected (e.g., 10CQ = 2010 collection year).

Wild Adults and Code	Total N	Analy	Par	Wild Juveniles and Code	Total N	Hatchery Adults and Code	Total N
Finney Creek	**41**	**0**	**0**	**Bacon Creek**	**48**	**Marblemount**	**117**
10CQ	19	0	0	07MS	2	08LF	22
11BK	22	0	0	10BA	46	09CF	51
middle Skagit River	**35**	**0**	**0**	**lower Cascade River**	**79**	10AN	44
09BM	9	0	0	09EE	39	**Bogachiel**	**136**
10AS	26	0	0	10AV	40	08AK	136
Sauk River	**82**	**17**	**20**	**County Line Ponds**	**54**		
08DR	5	0	0	07MU	5		
08MS	3	0	0	08MK	5		
09DU	13	0	0	09IY	9		
10AR	17	0	0	10BB	35		
11BN	15	3	7	**Diobsud Creek**	**39**		
81AAA	5	3	1	10BK	39		
83AAA	24	11	12	**Finney Creek**	**91**		
Suiattle River	**44**	**16**	**23**	09EH	45		
10AQ	15	7	8	10AT	46		
11BM	29	9	15	**Goodell Creek**	**61**		
upper Skagit River	**55**	**27**	**17**	09IZ	25		
08DQ	2	0	0	10BC	36		
09BN	5	4	1	**Grandy Creek**	**61**		
10AO	22	10	9	09EI	17		
11BI	26	13	7	10AY	44		
				Sauk River	**23**		
				10AX	23		
				Suiattle River	**56**		
				10AW	56		
				upper Skagit River	**50**		
				07MT	1		
				10AZ	49		

10.2.1 Simulation and Model

I used a simulation and an empirical model to construct datasets each with genetic diversity and hatchery-wild differentiation parameters similar to those documented among steelhead populations (i.e., spawning aggregates) in the Skagit River basin. The advantage of using simulated data is that all individuals are identified as being from known groups (e.g., pure "hatchery" and "wild", and their F1 hybrids), enabling an assessment of the power the genetic markers provide and the efficacy of our methods to assign individuals to these known groups. The difference between the simulated and the empirical model datasets is that for the simulated data all individuals were computer-generated (in silico) while for the empirical model I used steelhead individuals from the original dataset (Table 29) and their genotypes to create F1 hybrid offspring.

I simulated population allele frequencies for 15 microsatellite loci and 1000 chromosomes (i.e., haploids or "gametes" from 1000 individuals) using the program MS (Hudson 2002) and implemented with the command line: *./ms 1000 15 -t 14 -I 2 500 500 19 | ./microsat > msat.txt*. For each of the 15 independently evolving loci MS constructed a coalescent tree that approximates evolution under a Wright-Fisher model (*sensu* Crow and Kimura 1970) based on the number of user-defined chromosomes (e.g., 1000). Mutations were then placed onto the coalescent tree based on a user-defined mutation parameter (-t 14), which is equal to a mutation rate scaled by diploid effective population size (Hudson 2002). Varying the mutation parameter affects genetic diversity within the simulated population; after several trials I settled on the mutation parameter (14), which generated an average number of alleles per locus of 15.67 (see below). The output from MS is a series of 0s and 1s for each locus representing mutations at random sites along each chromosome. I then used the program MICROSAT (provided with MS) to convert the MS output to a series of one-step mutations that represent microsatellite alleles. Since the goal of this simulation was to generate a dataset that would approximate a realistic steelhead hatchery-wild dataset from the Skagit River, I used MS and MICROSAT to create two populations differentiated by F_{ST} = 0.05, and each represented by 500 chromosomes (haploid individuals) (-I 2 500 500 19 in the above MS command). To produce an F_{ST} = 0.05, I set the migration parameter to 19 following Anderson and Dunham (2008). From these steps I produced a dataset that consisted of allele frequencies for 15 microsatellite loci for two populations constructed from 500 chromosomes (haploid individuals) each. I refer to this dataset as "Simulated Populations."

From the alleles frequencies in the Simulated Populations dataset I used a custom Perl script to create 200 diploid individuals from each population. Of these, 100 individuals from each population were designated "parents," which I then used to produce 100 F1 hybrid individuals (i.e., 100 monogamous "mating" each producing a single offspring). My final simulated dataset ("Simulated Samples") consisted of the 100 non-parents from each population, and 100 unrelated F1 hybrids, for a total 300 individuals from three groups: Population 1, arbitrarily designated as the "hatchery", Population 2, designated as "wild," and F1 hybrids representing introgressive hybrids between the segregated hatchery and wild populations.

To determine if an increase in the number of loci would increase the power to detect hybrids, I altered the simulation described above to generate 500 microsatellite loci, instead of 15 loci (*./ms 1000* ***500*** *-t 14 -I 2 500 500 19 | ./microsat > msat.txt*.). I then selected randomly from the 500 loci, 100 and 15 locus-sets, producing three new data sets consisting of 300 individuals each from three groups: Population 1 (“hatchery”), Population 2 (“wild,”), and F1 hybrids, genotyped at 500, 100, and 15 randomly selected loci, respectively.

10.2.2 Empirical Model

In order to obtain the empirical data for simulations, I conducted a preliminary assessment as to the status (hatchery, wild, or introgressed hybrid) of each of the 374 adult samples (Table 1, wild adults [N = 257], Marblemount Hatchery [N = 117]) using the program STRUCTURE (Pritchard et al. 2000, Falush et al. 2003), and ranked each wild individual in terms of their wild purity (see below regarding STRUCTURE methods and results), and selected the 120 most “pure” Skagit River wild fish, regardless of their spawning area. As with the simulated populations described above, I randomly designated half of the individuals (60) as “parents” and the other half to be used in the analysis (Table 29). Wild Skagit River “parents” were “spawned” with 60 randomly selected fish from the Bogachiel Hatchery (see below) by randomly selecting one allele from each parent for each locus to produce 60 F1 hybrid individuals. Bogachiel Hatchery is located on the Bogachiel River, a tributary of the Quileute River, Washington Coast, and as with the Marblemount Hatchery, the origin of its winter-run steelhead broodstock is Chambers Creek. There is little genetic differentiation between broodstock from Marblemount and Bogachiel hatcheries (WDFW, unpublished data). However, although both hatcheries are managed as segregated programs, I selected Bogachiel Hatchery individuals for this model to reduce the chance that genetic similarities between the hatchery and wild populations in the Skagit Basin resulted, in part, from hatchery practices (i.e., using wild individuals as broodstock). That is, while there is a small but greater than zero probability that wild Skagit fish were used as broodstock in the Marblemount Hatchery, there is near-zero probability that wild Skagit fish were used as broodstock in the Bogachiel Hatchery. As with the simulated dataset, I constructed the empirical model dataset using 60 non-parents each from Skagit wild and Bogachiel Hatchery (Table 29), and 60 F1 hybrids, for a total 180 individuals from three groups.

10.2.3 Decreasing the Proportion of F1 Hybrids

The simulation and empirical models are based on a hybrid to wild fish ratio of 1:1 (true hatchery introgression = 50%). To determine if the true hatchery introgression rate affects the assignment error rate, for both the simulation and empirical model, I decreased the proportion of F1 hybrids to 25%, 10%, 5%, and 2% by randomly selecting, without replacement, F1 hybrid individuals from the analysis, after I assigned individuals using STRUCTURE. For example to achieve a 25% F1 hybrid proportion I randomly selected 33 of the 100 F1 hybrids (and their Q-values from the STRUCTURE analysis), and recalculated assignment error rates using these 33 F1 hybrids and the original 100 wild individuals. Since the original F1 hybrid samples sizes were small (100 and 60 for the simulation and empirical model, respectively), the low F1 hybrid proportions used very few F1 hybrid individuals in the analysis. For example, for the 2% F1 hybrid proportion I randomly selected only two and one F1 hybrid individuals from the 100 and 60 original set, for the simulation and empirical model, respectively. I repeated this procedure 10,000 times to produce estimates of 10,000 error rates each for the 25%, 10%, 5%, and 2% F1 hybrid proportions, for both the simulation and empirical model.

10.2.4 Statistical Analyses

The program STRUCTURE is one of the most widely used programs for inferring population structure (see Gilbert et al. 2012 for summary of its use), and has also been used for detecting hybrid individuals, frequently between wild and domestic populations (e.g., Norén et al. 2005, Kidd et al. 2009, Sanz et al. 2009, Marie et al. 2011, Lamaze et al. 2012). The program STRUCTURE makes use of each individual's multilocus genotype, such as microsatellites, to infer population structure (e.g., hatchery versus wild), given an *aprioi* inferred number of groups or populations (here, two populations: hatchery and wild). The program will probabilistically assign individuals to populations, or if the admixture option is used, will assign a portion of an individual's genotype to populations (Pritichard et al. 2000). I used STRUCTURE for analyses of both the simulation and empirical model populations, and to investigate introgressive hybridization within Skagit River steelhead populations using empirical (non-modeled) data. I used the following parameters to identify pure hatchery, pure wild, and F1 hybrids in all datasets: 50,000 burn-in steps and 500,000 MCMC steps; admixture model, correlated allele frequencies, and 90% credible interval. All STRUCTURE runs used K=2 (K = inferred numbers of genetic groups, here representing hatchery and wild) and were repeated using 6-10 iterations each. When using the admixture option the program STRUCTURE does not explicitly assign individuals to specific groups (here, hatchery or wild), but will provide an estimate of the proportion of an individual's genome (Q-value) that can be assigned to each group. Within a single run, these Q-values are the posterior mean estimates of these proportions. *A priori*, one would expect pure hatchery or wild individuals to have Q-values with a high proportion to one group and low proportion to the other group (e.g., for a pure hatchery individual, a hatchery Q-value → 1.00 and a wild Q-value → 0.00), and a F1 hybrid individual to have intermediate and equal Q-values (hatchery and wild Q-values are equal and approximately 0.50). For each iteration of STRUCTURE, the program reports the 90% credibility interval (CI), the middle 90% of the distribution of Q-values throughout the 500,000 MCMC steps. The CI measures the uncertainty of the reported Q-value, with broad ranges signaling greater uncertainty than narrow ranges. Therefore, the CI provides a measure of confidence of the final estimate of the hatchery and wild proportions for each individual's genome (i.e., Q-value). I used MATLAB (version 8.0.0.783 (R2012b), The MathWorks, Natick, Massachusetts) or Perl for all ancillary analyses and to produce all graphics.

10.3 Results

10.3.1 Veracity of Simulated Dataset

The analysis of the simulated data is only useful if the data appropriately mirror the genetic diversity and hatchery-wild differentiation of the steelhead populations within the Skagit River basin. Although the simulated and empirical model datasets showed slightly lower numbers of alleles per locus than the All Adult or Complete Adult datasets (15 and 14.5, compared with 17.3 and 16.3, respectively), their gene diversity measures were either greater (0.89, Simulated Samples) or the same (0.81, Empirical Model) as the those in the observed datasets (Table 30). Similarly, pairwise population differentiation was the same in the observed and simulated datasets (0.02), but slightly greater in the empirical model dataset (0.04, Table 30). The amount of genetic differentiation and diversity within the simulated and model datasets compared well with the genetic variation observed within the Skagit River basin steelhead populations and the differentiation between Marblemount Hatchery and Skagit River wild populations. Therefore both the simulated and empirical model datasets are appropriate as models for the Skagit River basin steelhead populations.

Table 30. Genetic diversity and differentiation parameters for five datasets. All Adult Samples (from Kassler and Warheit 2012) and Complete Adult Samples (from Table 1) include only the adult samples from Finney Creek, middle Skagit River, Sauk River, Suiattle River, upper Skagit River, and Marblemount Hatchery. All other datasets include only one hatchery and one wild population. The Simulated Samples were drawn from the Simulated Populations, as discussed in the Methods section, and the Empirical Model populations are shown in Table 29 including 60 samples from Bogachiel Hatchery. Gene diversity is expected heterozygosity (Weir 1996). Population differentiation (theta or F_{ST}, Cockerham 1969) for the All Adult Samples and Complete Adult Samples are averages of five pairwise theta measures between wild populations and Marblemount Hatchery. Theta for the Simulated Populations was not calculated but the populations were designed to have a theta = 0.05.

Population Parameter	All Adult Samples	Complete Adult Samples	Simulated Populations	Simulated Samples	Empirical Model
Sample size	536 diploids	374 diploids	1000 haploids	200 diploids	120 diploids
Number populations	6	6	2	2	2
Alleles per locus	17.3	16.3	15.67	15	14.5
Gene diversity	0.81	0.81	n/a	0.89	0.81
Theta (F_{ST})	0.02	0.02	0.05 (modeled)	0.02	0.04

10.3.2 Power to Detect Hybrids-Simulation

Although most hatchery (Population 1) individuals have high hatchery Q-values and most wild (Population 2) individuals have low hatchery Q-values, the range of Q-values for both groups overlap (Figure 24). Furthermore, the hatchery Q-values for the F1 hybrid individuals are not clustered around 0.50, as you would expect, but show an even distribution ranging as low as 0.05 and as high as nearly 1.00 (Figure 24). Under these conditions, assigning individuals to a particular group (i.e., hatchery, wild, or F1 hybrid) required that Q-value limits or thresholds be used to define groups. I defined arbitrarily the limit for the hatchery group as any hatchery Q-value equal to or greater than 0.80, and conversely, the wild group as any hatchery Q-value equal to or less than 0.20. [For k = 2 analyses the Q-values are complementary; that is, by definition an individual with a hatchery Q-value = 0.80 would have a wild Q-value = 0.20]. For both the hatchery and wild populations, 84 of the 100 simulated individuals had hatchery Q-values greater than 0.80 and less than 0.20, respectively, and therefore would be correctly assigned as either a pure hatchery or wild fish (Table 31).

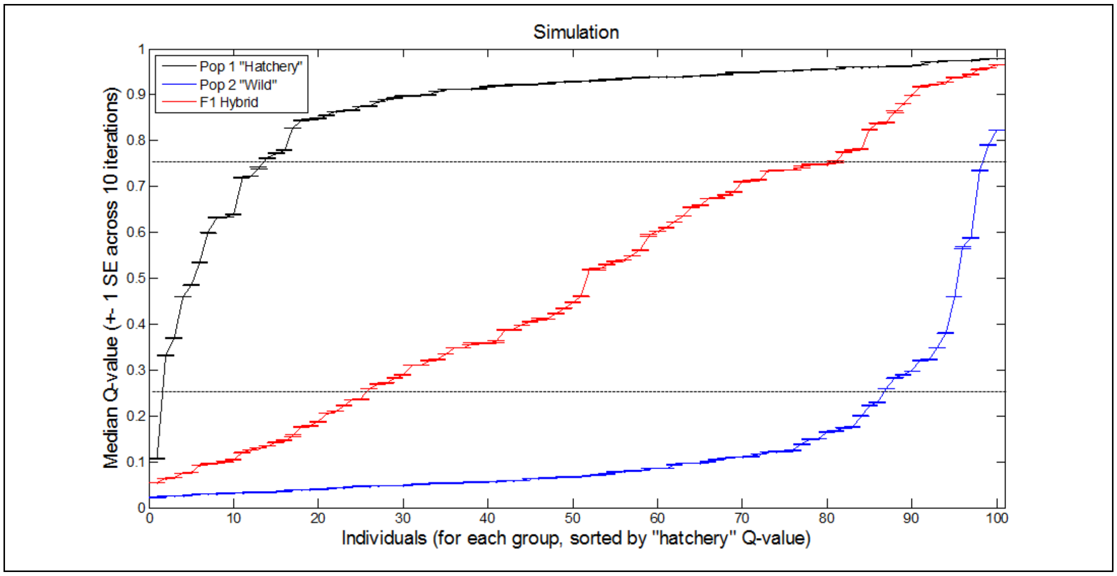


Figure 24. Results from STRUCTURE analysis using simulated populations. Plot represents two populations and their F1 hybrids. No parents were included in the analysis. Dataset was subjected to ten iterations of STRUCTURE (k=2) with points (not visible, but intersection between curves and horizontal lines along curves) corresponding to median scores and horizontal lines defining ±1 SE (1 SE for each point is small so error bars appear as a single horizontal bar). Individuals for each population (and F1 hybrids) are sorted separately by their Q-value for Population 1 (roughly equivalent to percent of genome from Population 1). Here, Population 1 was arbitrarily designated as the "hatchery" group. Dotted black horizontal lines show approximately the Q= 0.25 and 0.75 interval.

Table 31. Proportion (top) and median range of the 90% credibility interval (bottom) of simulated hatchery (Pop 1), wild (Pop 2), and F1 Hybrid individuals assigned as wild, hatchery, or hybrid (introgressed), or as unidentified, based on the Q-value thresholds of <= 0.20 for wild, >=0.80 for hatchery, and >0.40 - <0.60 or >0.25 - <0.75 for introgressed individuals. The proportion unidentified depends on the thresholds for the introgressed hybrids.

	Median Hatchery Q-value across 10 iterations						
Source	<= 0.20	>=0.80	>0.40- <0.60	>0.25- <0.75	>0.40- <0.60	>0.25- <0.75	*Total Count*
	Wild	Hatchery	Introgressed		Unidentified		
Pop1 (Hatchery)	0.01	0.84	0.04	0.12	0.11	0.03	*100*
Pop2 (Wild)	0.84	0.01	0.03	0.12	0.12	0.03	*100*
F1_Hybrid	0.20	0.16	0.15	0.55	0.49	0.09	*100*
Total Count	*105*	*101*	*22*	*79*	*72*	*15*	*300*
Pop1 (Hatchery)	0.57	0.37	1.00	1.00	0.99	0.97	
Pop2 (Wild)	0.39	0.97	1.00	1.00	0.99	0.96	
F1_Hybrid	0.59	0.47	1.00	1.00	0.99	0.93	

I evaluated two alternative threshold ranges for defining the F1 hybrid group: Q-values 0.40-0.60 and 0.25-0.75 (Table 31). Only 15 F1 hybrids had Q-values between 0.40 and 0.60, and would be assigned as a hybrid using this criterion, but that number increased to 55 when the threshold was expanded to Q-values between 0.25 and 0.75 (Figure 24, Table 31). If we consider only those fish that were assigned, the error for assigning pure hatchery or wild fish (i.e., number of *incorrect* assignments / total number assigned) ranged from 0.05 to 0.13, while the error for the F1 hybrids was between 0.40 and 0.71 (Table 32). Due to a broader range between the limits that define the hybrid and pure groups, the 0.40-0.60 hatchery threshold produced more unidentified fish than the 0.25-0.75 threshold. The 0.40-0.60 threshold resulted in relatively few assignments for the F1 hybrids (51% assigned), and for those individuals that were assigned only 29% were correctly identified as hybrids (Table 31 and 32).

Table 32. Assignment and error rates from STRUCTURE analyses for the simulated and empirical model datasets, using two threshold ranges for assigning F1 hybrids. For the 0.40-0.60 threshold range, individuals with Q-values between 0.40 and 0.60 were assigned as F1 hybrids. Likewise, for the 0.25-0.75 threshold range, individuals with Q-values between 0.25 and 0.75 were assigned as F1 hybrids. Individuals with Q-values falling in between the threshold ranges that define the hybrid and pure groups were not assigned to any group. Individuals not assigned to a group were removed from the analysis when calculating the proportion of fish correctly/incorrectly assigned. For example, 84 hatchery (Pop1) fish had hatchery Q-values greater than 0.80, while 11 fish had hatchery Q-values between 0.60 and 0.80, making them unassigned. The proportion correctly assigned is 84/89 or 0.94 as indicated. Results here correspond to the data presented in Figures 24 and 29 for the simulated and empirical model datasets, respectively.

Source Population	Model	Threshold Range	Assigned		Unidentified
			Correct	Incorrect	
Pop1 (Hatchery)	Simulation	≥ 0.80	0.94	0.06	0.11
Pop2 (Wild)	Simulation	≤ 0.20	0.95	0.05	0.12
F1_Hybrid	Simulation	0.40-0.60	0.29	0.71	0.49
Pop1 (Hatchery)	Simulation	≥ 0.80	0.87	0.13	0.03
Pop2 (Wild)	Simulation	≤ 0.20	0.87	0.13	0.03
F1_Hybrid	Simulation	0.25-0.75	0.60	0.40	0.09
Chambers (Bogachiel)	Skagit Empirical	≥ 0.80	1.00	0.00	0.05
Skagit Natural	Skagit Empirical	≤ 0.20	0.98	0.02	0.03
F1_Hybrid	Skagit Empirical	0.40-0.60	0.31	0.69	0.47
Chambers (Bogachiel)	Skagit Empirical	≥ 0.80	0.95	0.05	0.00
Skagit Natural	Skagit Empirical	≤ 0.20	0.97	0.03	0.02
F1_Hybrid	Skagit Empirical	0.25-0.75	0.61	0.39	0.07

Because hatchery-origin steelhead within the Skagit River basin are marked by the removal of their adipose fin, to evaluate introgressive hybridization between hatchery and wild populations field crews would examine only those fish with intact adipose fins (i.e., natural-origin fish). Therefore, for simulated data, to evaluate assignment error I considered only the wild (Population 2) and F1 hybrid individuals. When I pooled the wild and F1 hybrids into a single mixed-origin collection, as they would occur naturally within the Skagit Basin, I assigned to either the wild or hybrid group 139 and 188 individuals using the 0.40-0.60 and 0.25-0.75 Q-value ranges, respectively, out of the original 200 simulated individuals (Table 33). Of these individuals, I assigned 51 and 91 of the hybrids, and 88 and 97 of the pure wild individuals. However, only 18 and 67 total individuals were assigned as hybrids using the 0.40-0.60 and 0.25-0.75 Q-value ranges, respectively, and 121 individuals were assigned as pure wild (Table 33). If I defined introgressive hybridization as the number of hybrid assignments divided by total assignments I would estimate hatchery introgression for this simulated dataset as being 13% (18/139) for the 0.40-0.60 range and 36% (67/188) for the 0.25-0.75 range, while the actual hatchery introgression for this dataset was 37% (51/139) and 48% (91/188), respectively (Table 33), including 16 F1 hybrids that were assigned to the pure hatchery group[2]. Error rates ((estimated introgression – actual introgression) / actual introgression) were -0.65 and -0.25 for the 0.40-0.60 range and 0.25-0.75 range, respectively, indicating that I underestimated introgression by 65% or 25%, depending on which hybrid Q-value range I used (the negative sign for these error rates indicates that the estimates were an underestimate, not that the error was below zero) (Table 33). The absolute value of the error rate for the 0.40-0.60 range was 2.6 times that of the 0.25-0.75 range.

Table 33. Errors for assigning individuals as hybrids. Except for the Actual Hatchery Introgression results, these calculations consider only the pure wild and F1 hybrid fish since at Marblemount Hatchery outmigrating juvenile fish are visibly marked with an adipose fin clip and would therefore not be confused as a natural-origin fish when they return as adults. The Actual Hatchery Introgression results include 16 and 19 F1 hybrid individuals assigned as pure hatchery for the Simulation and Empirical Model, respectively. Total Number Individuals Assigned as Hybrid includes both pure wild and F1 hybrid fish with Q-values within the hybrid-defined ranges (either 0.40-0.60 or 0.25-0.75), and Total Number Individuals Assigned as Pure Wild includes both pure wild and F1 hybrid fish with Q-values <= 0.20. See text for error rate calculations and Figures 24 and 29 for Q-value distributions for F1 hybrid and pure wild individuals. A negative error rate indicates an under-estimation of the number of hybrids.

	Simulation		Skagit - Empirical Model	
	0.40-0.60	0.25-0.75	0.40-0.60	0.25-0.75
Total Number Individuals	200	200	120	120
Number Individuals Assigned	139	188	90	115
Number Simulated/Modeled Pure Wild Assigned	88	97	58	59
Number Simulated/Modeled Hybrids Assigned	51	91	32	56
Total Number Individuals Assigned as Pure Wild	121	121	79	79
Total Number Individuals Assigned as Hybrid	18	67	11	36
Error Assigning Individuals as Hybrids	0.65	0.26	0.66	0.36
Error Assigning Individuals as Pure Wild	0.38	0.33	0.36	0.34
Estimated Hatchery Introgression	0.13	0.36	0.12	0.32
Actual Hatchery Introgression	0.37	0.48	0.36	0.49
Error Rate (Estimate – Actual) / Actual	-0.65	-0.25	-0.67	-0.35

[2] The simulated hatchery introgression is actually 0.50 (i.e., 100 simulated pure wild and 100 simulated F1 hybrids). However, I based my calculation on the number of assigned individuals, removing from the analyses the unidentified individuals. Since the number of unidentified F1 hybrids is greater than that for the pure wild fish, hatchery introgression among the assigned individuals is less than 0.50.

Hatchery and wild individuals that are incorrectly classified have larger 90% credibility intervals (CIs) than those that are correctly classified (Q >= 0.80 for hatchery and Q <= 0.20 for wild), but the opposite is true for the F1 hybrids (Figure 25). That is, F1 hybrid individuals that were correctly classified as hybrids showed CIs that ranged from 0 to 1.00, but when individuals were incorrectly classified as either wild or hatchery their CI ranges were 0.59 and 0.47, respectively (Table 31). In contrast, the CI ranges for pure hatchery and wild individuals classified as hybrids were 1.00, but their ranges for correct assignments were 0.37 and 0.39, respectively (Table 31). Even though the CIs for F1 hybrids identified as hatchery (Q-value >= 0.80) or wild (Q-value <= 0.20) were smaller than those for individuals correctly identified a hybrid, these CIs were still larger than those for correctly identified hatchery and wild individuals

(Table 31, Figure 26). Individuals identified as either hybrids or not identified showed CI ranges equal to or nearly equal to 1.00, regardless of their source, indicating that there was low confidence in assigning individuals when the Q-values are greater than 0.20 and less than 0.80 (Figure 27).

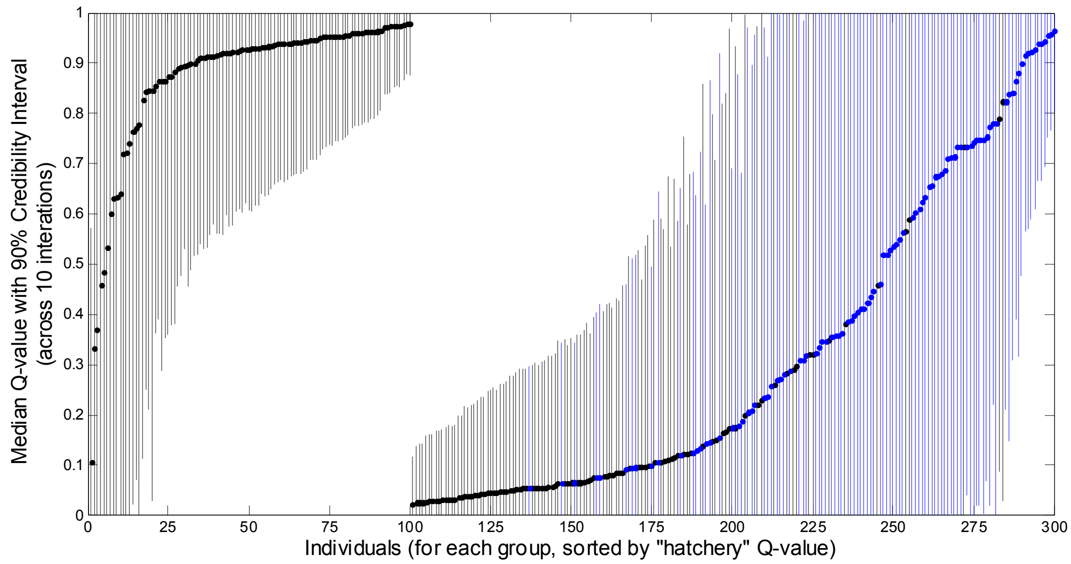


Figure 25. As in Figure 30, except each individual is now represented by its Population 1 (hatchery) Q-value (point) and the 90% credibility interval for that Q-value (bar). The Q-value and the end points for the 90% CI are median values from the ten iterations. Individuals 1-100 are from Population 1("hatchery"), and individuals 101 – 300 are combined Population 2 ("wild") and F1 hybrids (blue). All individuals with the two groups are sorted by "hatchery" Q-values.

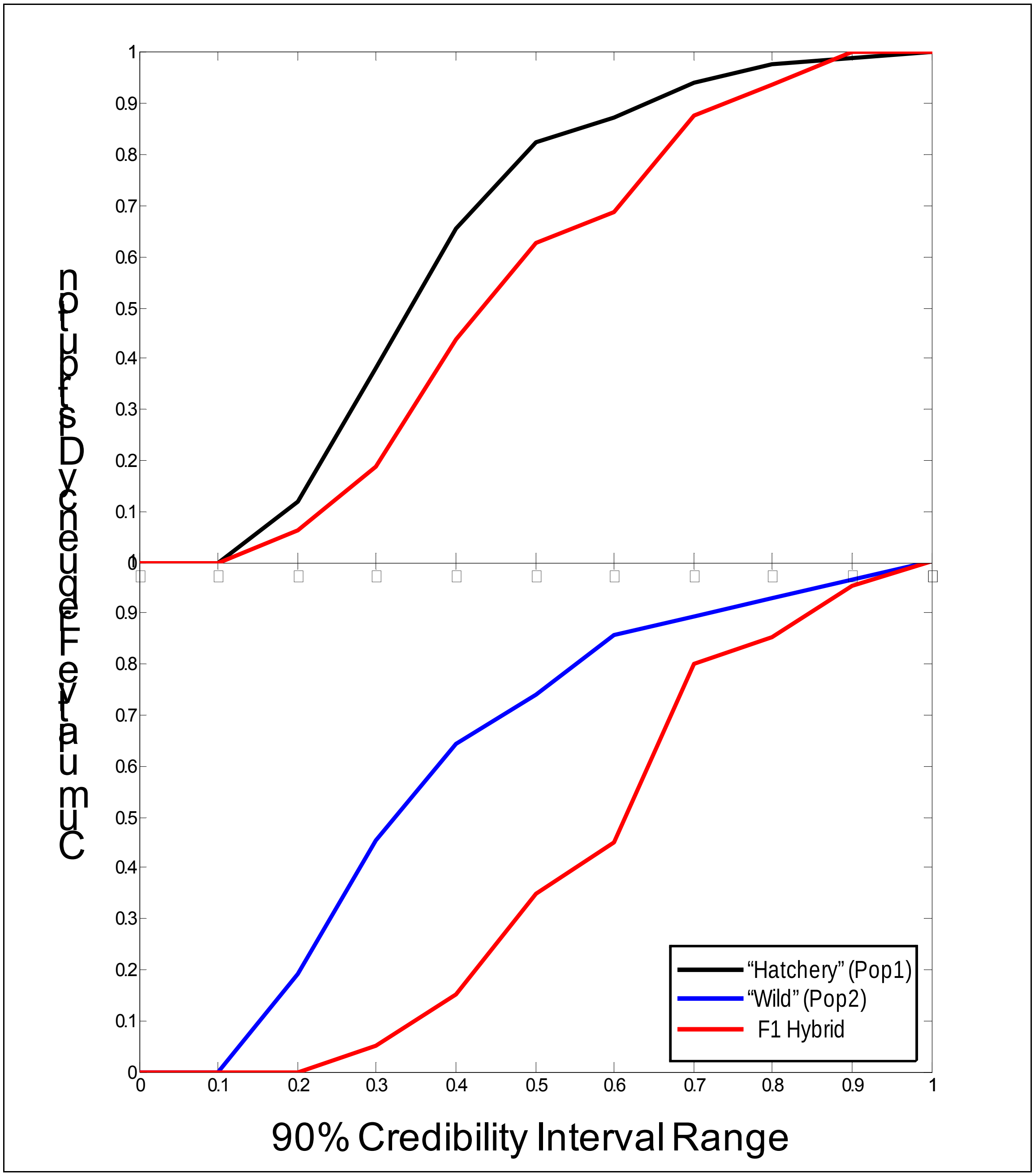


Figure 26. Cumulative frequency distribution for the 90% Credibility Interval range for all simulated individuals whose Q-value ≥ 0.80 (top, “hatchery”) or ≤ 0.20 (bottom, “wild”). Both plots show larger ranges, and therefore more uncertainty, for assigning F1 hybrids as pure “hatchery” or “wild”, than the pure individuals. See Table 31.

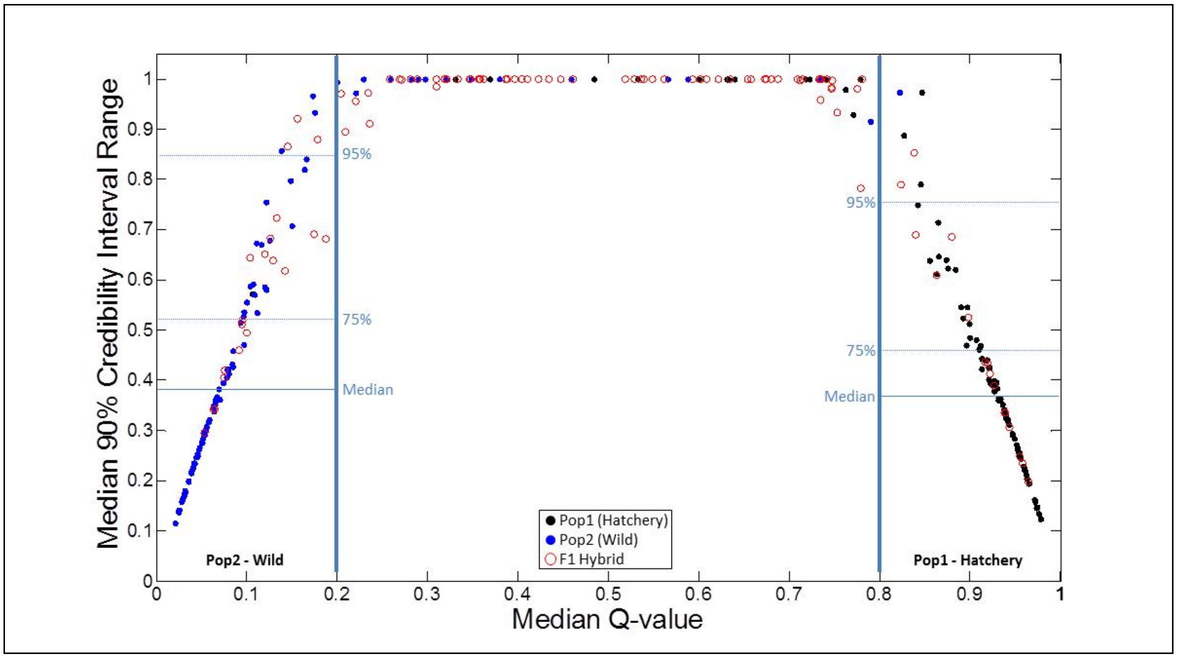


Figure 27. 90% Credibility Interval (CI) range as a function of Q-value for each of the 300 simulated individuals. Points represent median values across 10 STRUCTURE iterations. Also shown are the values for the median, and 75th and 95th percentiles for the distribution of median 90% CI ranges of correctly identified wild (left, Q <= 0.20) and hatchery (right, Q >= 0.80) individuals.

Overall, there is greater uncertainty in assigning F1 hybrid individuals than pure individuals; however, there are individual F1 hybrids with CI ranges as short as some the shortest CI ranges of correctly assigned hatchery and wild individuals (Figure 27). For F1 hybrids with Q-values >= 0.80 (N = 16), 88% had CI ranges within the 95th percentile of the CI ranges for correctly assigned hatchery individuals, 63% within the 75th percentile, and 38% below the CI range median for correctly assigned hatchery individuals (Figure 27). There is similar pattern for CI ranges of F1 hybrids assigned as pure wild (Q-values <= 0.20), compared with the CI ranges for correctly assigned wild individuals (Figure 27). As such, individuals with Q-values within the range of pure hatchery (Q >= 0.80) or wild (Q <= 0.20), and with relatively short CI ranges cannot be identified with confidence as either pure hatchery or wild, respectively because hybrid individuals within these ranges have similar Q-value – CI range joint distributions.

10.3.3 Increasing Number of Loci - Simulation

Increasing the number of scored loci from 15 to 100 and 500 (assuming 15 alleles per locus: 1,500 and 7,500 alleles, respectively) increased the number of identified and correctly assigned individuals, resulted in smaller 90% CIs for each of the hatchery, wild, and F1 hybrid groups (Figure 28), and reduced or eliminated error in estimated introgression. Estimated introgression error rates were -0.55, -0.06, and 0.00 for the 15, 100, and 500 locus datasets using the 0.40-0.60 Q-value range, and -0.17, -0.04, and 0.00 using the 0.25-0.75 Q-value range. Except for the 500 locus datasets, where there was no error, the other datasets underestimated the number of hybrids, although for the 100 locus dataset the underestimation was only 4-6%.

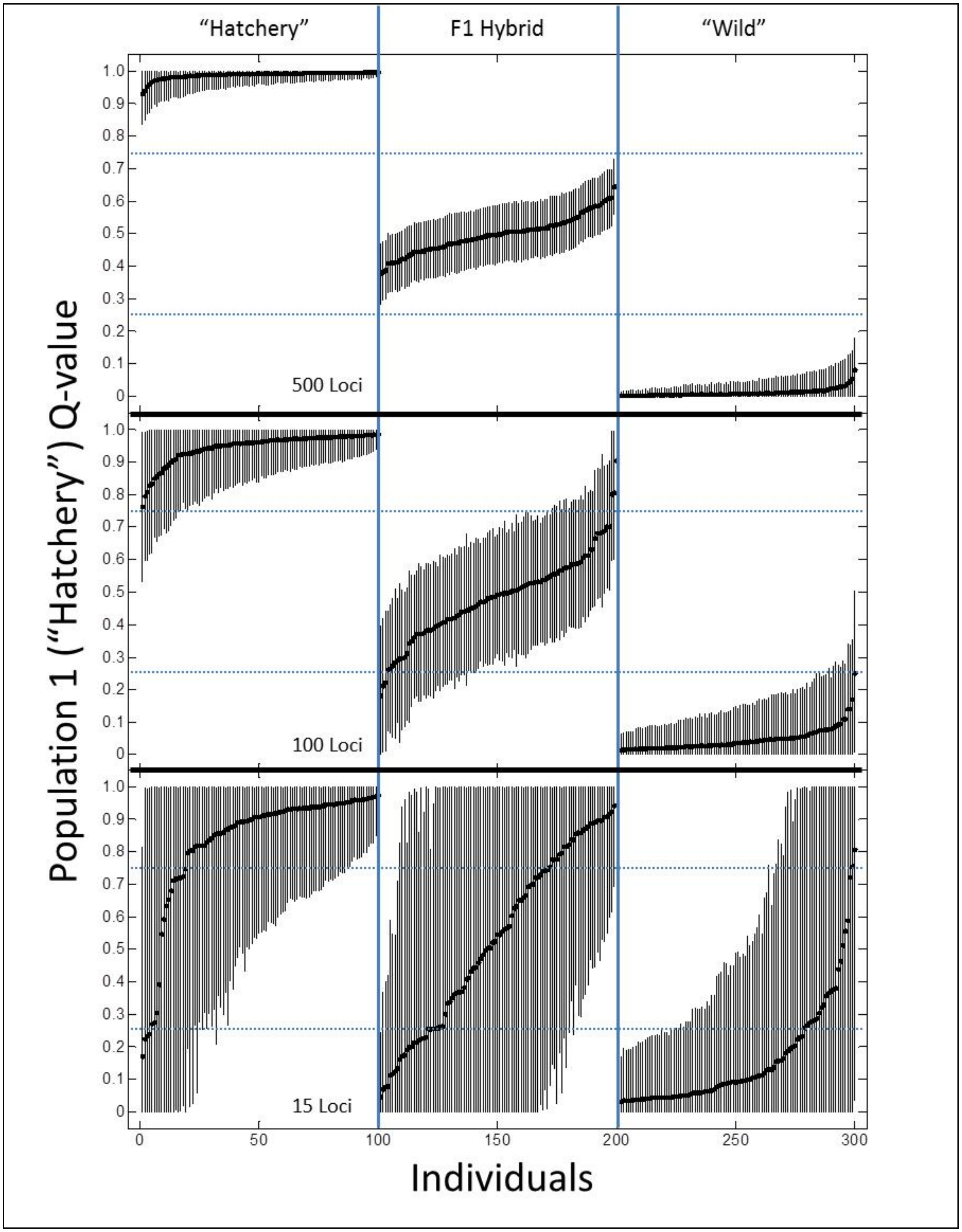


Figure 28. Hatchery Q-values (point) with their 90% credibility intervals (bar) for simulated individuals scored at 15 (bottom), 100 (middle), and 500 (top) loci. Individuals 1-100 are pure hatchery, 101-200 F1 hybrids, and 201-300 pure wild. All individuals are sorted by “hatchery” Q-values. For each plot, the 0.25-0.75 Q-value range is marked with dotted blue lines. The number of loci in the 15 locus set (bottom panel) is equivalent to that in the currently used SPAN microsatellite set.

10.3.4 Power to Detect F1 Hybrids – Empirical Model

Results for the empirical model are similar to those for the simulation. As with the simulation, the hatchery Q-values for the F1 hybrids ranged from nearly 0.1 to 1.0, but unlike the simulation their Q-value distribution appeared skewed towards the hatchery-origin (Figure 29), and a greater proportion of the F1 hybrid individuals were identified as pure hatchery (Bogachiel Hatchery) than pure wild (Skagit Natural) (Table 34). In addition, the Q-values for the pure hatchery and wild individuals were more restricted and without overlap in the empirical model than in the simulation (compare Figures 30 and 34). That is, there were no Bogachiel Hatchery individuals identified as Skagit Natural, and no Skagit Natural individuals identified as Bogachiel Hatchery (Table 34). The proportion of F1 hybrids that were assigned as hybrids in the empirical model was nearly identical to that in the simulation (0.17 and 0.57 for the 0.40-0.60 and 0.25-0.75 Q-value ranges, respectively, in the empirical model compared with 0.15 and 0.55 for that in the simulation; Tables 31 and 34). Since the proportion of individuals unassigned was similar between the empirical model and the simulation, the proportion of F1 hybrid individuals that were incorrectly assigned was also similar between the empirical model and the simulation (Table 32). As with the simulation, the STRUCTURE analysis using the empirical model dataset underestimated the proportion of hybrid individuals and therefore the extent of hatchery introgression, and the error rate was considerably larger for the 0.40-0.60 Q-value range than the 0.25-0.75 range (-0.67 and -0.35, respectively; Table 33).

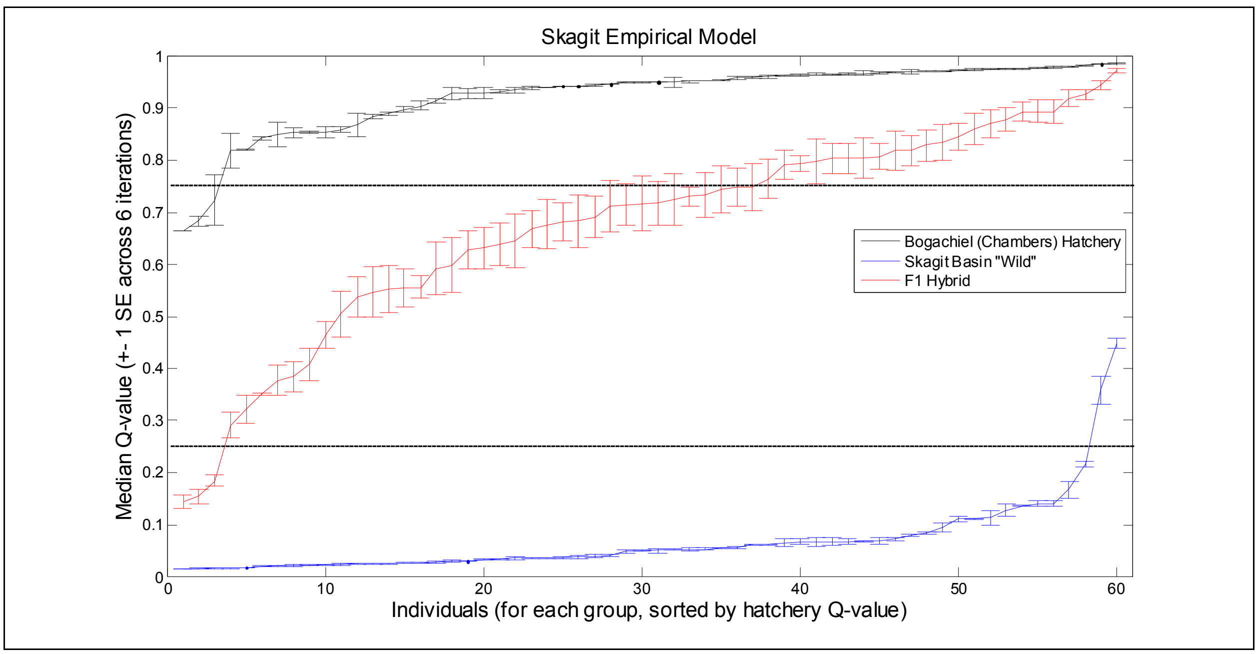


Figure 29. As in Figure 24, except STRUCTURE analysis (six iterations) was based on an empirical model using a collection of "pure" natural-origin individuals from the Skagit Basin and broodstock from Bogachiel Hatchery (Washington coast), representing Chambers-origin hatchery. As with the simulation shown in Figure 24, no parents were included in the analysis

Table 34. Proportion (top) and median range of the 90% credibility interval (bottom) of Bogachiel Hatchery, Skagit Natural, and F1 Hybrid individuals from the empirical model assigned as wild, hatchery, or hybrid (introgressed), or as unidentified, based on the Q-value thresholds of <= 0.20 for Skagit Natural, >=0.80 for Bogachiel Hatchery, and >0.40 - <0.60 or >0.25 - <0.75 for introgressed individuals. The proportion unidentified depends on the thresholds for the introgressed hybrids.

Source	Median Q-value across 10 iterations						*Total Count*
	<= 0.20	>=0.80	>0.40-<0.60	>0.25-<0.75	>0.40-<0.60	>0.25-<0.75	
	Wild	Hatchery	Introgressed		Unidentified		
Chambers (Bogachiel)	0.00	0.95	0.00	0.05	0.05	0.00	*60*
Skagit Natural	0.95	0.00	0.02	0.03	0.03	0.02	*60*
F1_Hybrid	0.05	0.32	0.17	0.57	0.47	0.07	*60*
Total Count	*60*	*76*	*11*	*39*	*33*	*5*	*180*
Chambers (Bogachiel)	-	0.38	1.00	1.00	1.00	-	
Skagit Natural	0.32	-	1.00	1.00	1.00	1.00	
F1_Hybrid	0.90	0.79	1.00	1.00	1.00	0.99	

For those individuals that were assigned as hatchery or wild, there was greater uncertainty in that assignment for F1 hybrids than for the pure individuals. F1 hybrid 90% CI ranges were large for most individuals (Figure 30), and as with the simulation, the CIs for F1 hybrids identified as either hatchery or wild were considerably larger than those for correctly identified hatchery and wild individuals (Table 34, Figure 31).

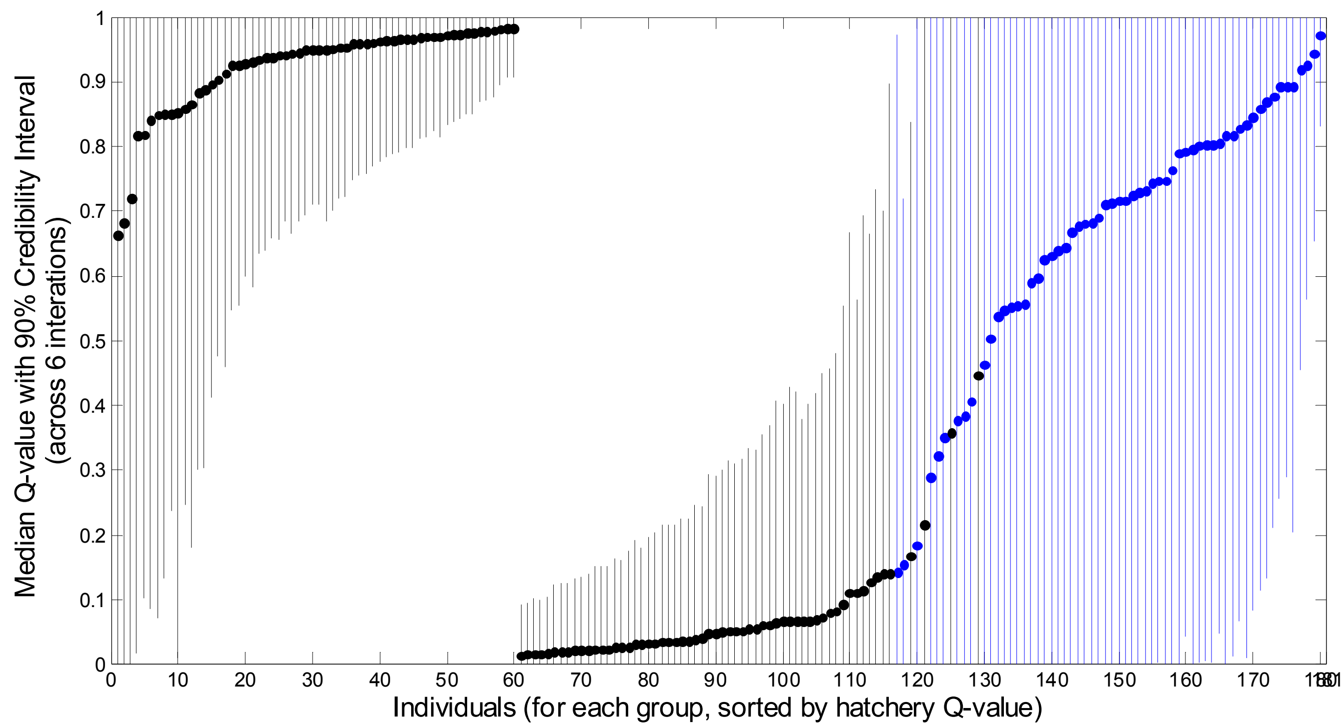


Figure 30. As in Figure 3, except STRUCTURE analysis (six iterations) based on empirical model (see Figure 29).

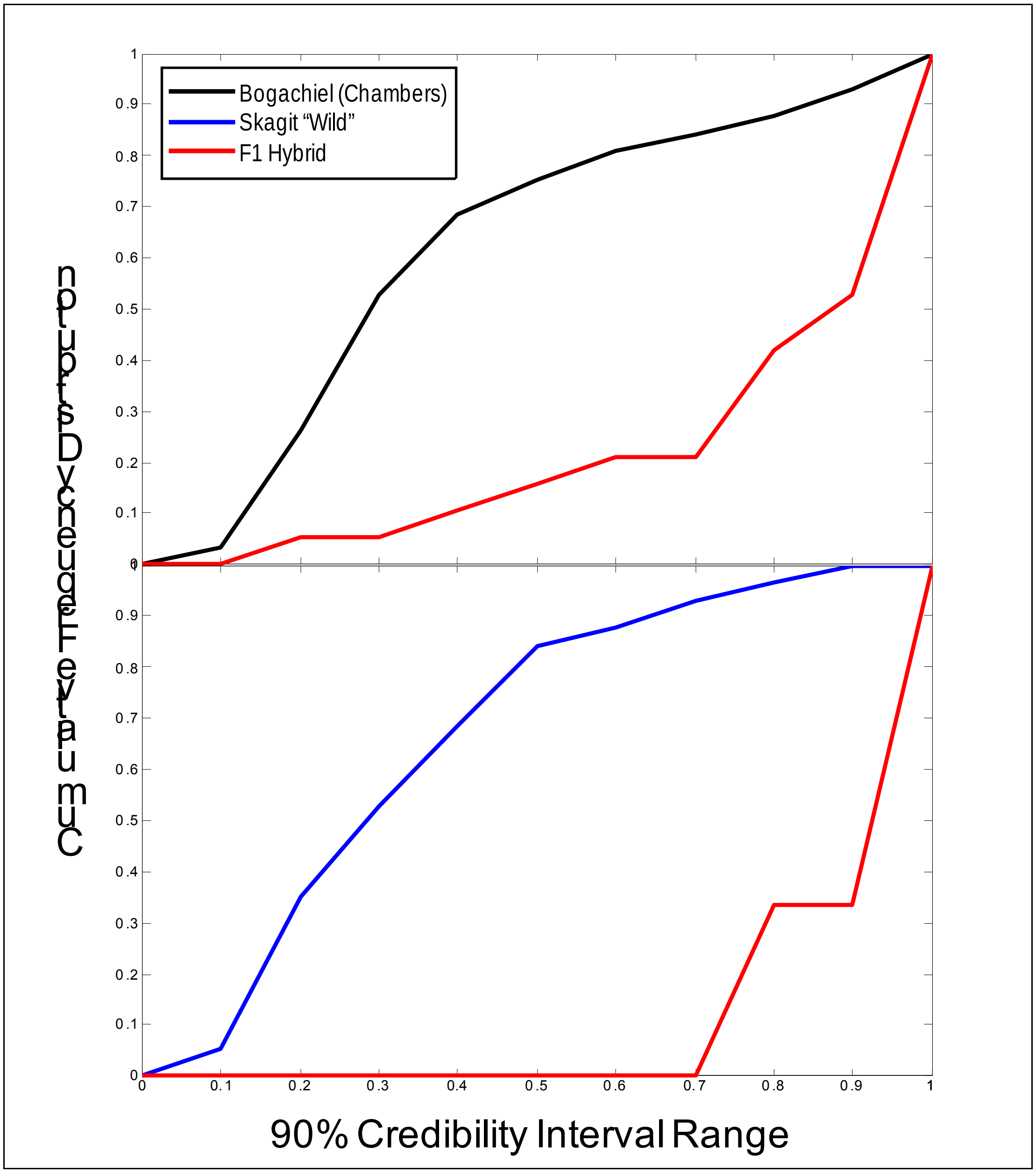


Figure 31. Cumulative frequency distribution for the 90% credibility interval range for all individuals from the empirical model whose Q-value ≥ 0.80 (top, "hatchery") or ≤ 0.20 (bottom, "wild"). Both plots show larger ranges, and therefore more uncertainty, for assigning F1 hybrids as pure "hatchery" or "wild", than the pure individuals. N = 5 for F1 hybrid in bottom plot. See Table 34.

10.3.5 Decreasing Proportion of F1 Hybrids

For these analyses I used only the 0.25-0.75 Q-value threshold to assign an individual as a hybrid. For both the simulation and empirical model, errors rates increased as the proportion of the F1 hybrids decreased, moving from an underestimate of 25% for 50% proportion (Table 33) to an overestimate of nearly 600% (14 individuals assigned as hybrids rather than the true value of 2 individuals) for the 2% proportion (median value, non-infinity values) for the simulation (Figure 32). For the empirical model the error rates were an underestimate of 35% for 50%

proportion (Table 33) and an overestimate of 200% for the 2% proportion (Figure 33). Furthermore, as the proportions decreased the error rate variance increased resulting in lower confidence of the estimated error rate for a proportion of 2% compared with a proportion of 25%. Error rates extended to infinity for the 2% proportion for the simulation (Figure 32) and for the 5% and 2% proportion for the empirical model (Figure 33).

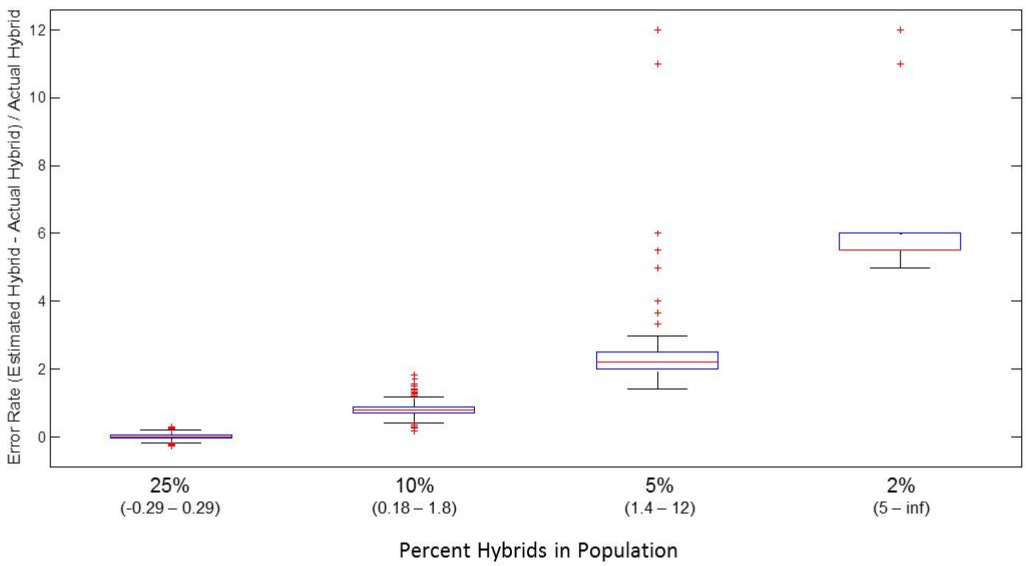


Figure 32. Distribution of error rates from 10,000 iterations each for four different F1 hybrid proportions (25% - 2%) from the simulation. For each box plot, the red line is the median, upper and lower edges of the box are the 75th and 25th percentiles, respectively, tips of the whiskers represent the 99% confidence interval (for a normal distribution), and the + are extreme values beyond the 99% CI. The parenthetical range of numbers below each F1 hybrid proportion label are the error rate ranges for each proportion. Negative error rates indicate an underestimate of the proportion of hybrid individuals, and likewise, positive error rates indicate an overestimate. The error rate range for the 2% proportion extended to infinity, but its boxplot is based on only the non-infinity values. Error rates extend from -29% (-0.29) to over 1200% (12).

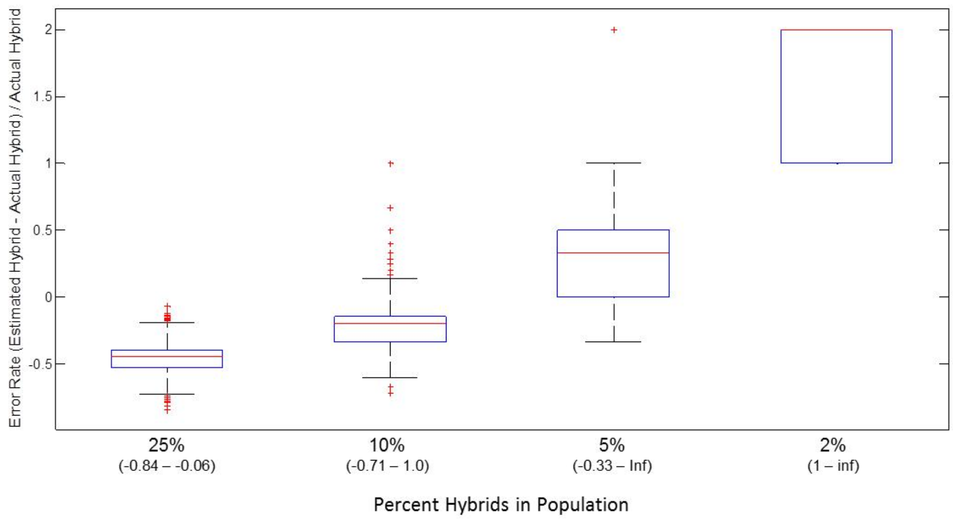


Figure 33. As in Figure 10, except for the empirical model. The error rate range for both the 5% and 2% proportions extended to infinity, but their boxplot are based on only the non-infinity values. Error rates extend from -84% (-0.84) to over 200% (2).

10.3.6 Empirical Analysis of Introgressive Hybridization within Skagit River Basin

Because the analysis of the simulated and modeled populations showed smaller error rates and greater number of individuals assigned for the 0.25-0.75 Q-value threshold than the 0.4-0.6 threshold (Table 5), I limited my analysis here to the 0.25-0.75 threshold. In addition, I measured introgression using both the Marblemount and Bogachiel hatchery samples, in separate analyses. Only 0.88 of the Marblemount Hatchery individuals assigned back to the hatchery group, although no incorrectly assigned individuals had Q-values <= 0.20 (Table 35, Figure 34). This compares with 0.94 of the Bogachiel Hatchery individuals that assigned back to the hatchery group, although here, 0.01 had Q-values <= 0.20 (Table 36, Figure 35). For all adult wild populations fewer individuals had hatchery Q-values >= 0.25 for the analysis using Bogachiel Hatchery, compared with Marblemount Hatchery, resulting in fewer wild individuals assigned to the Bogachiel Hatchery or introgressed groups (Tables 35 and 36, Figure 34 and 35). The occurrence of natural-origin introgressed adult individuals ranged 0.05-0.20 and 0.02-0.17 for the Marblemount and Bogachiel hatchery analyses, respectively. The Finney Creek population stands out among the adult populations as having consistently higher numbers of individuals with hatchery Q-values between 0.25 and 0.75 and therefore assigned to the introgressed group. This is particularly evident in the STRUCTURE analysis that used Bogachiel Hatchery samples (Figure 35). In addition, the Finney Creek, Sauk River, and upper Skagit River populations have individuals with Q-values >= 0.80 and with relatively small 90% CI ranges (Figure 35).

Table 35. Proportion (top) and median range of the 90% credibility interval (bottom) for the wild adult and Marblemount Hatchery populations assigned to the wild (median Q-value <=0.20), hatchery (median Q-value >=0.80), or introgressed (median Q-value >0.25 and < 0.75) group, or not identified to group. Although analysis was based on K = 2, individuals from all six populations were run simultaneously

Source	Median Q-value across 10 iterations				*Total Count*
	<= 0.20	>=0.80	>0.25 and <0.75		
	Wild	Hatchery	Introgressed	Unidentified	
Marblemount Hatchery	0.00	0.88	0.10	0.02	*117*
Finney Creek	0.71	0.10	0.20	0.00	*41*
middle Skagit River	0.83	0.06	0.11	0.00	*35*
Sauk River	0.87	0.05	0.05	0.04	*82*
Suiattle River	0.77	0.02	0.14	0.07	*44*
upper Skagit River	0.76	0.07	0.15	0.02	*55*
Total Count	*205*	*118*	*42*	*9*	*374*
Marblemount Hatchery	-	0.25	0.96	0.74	
Finney Creek	0.43	0.57	1.00	-	
middle Skagit River	0.36	0.89	1.00	-	
Sauk River	0.28	0.47	1.00	0.92	
Suiattle River	0.31	0.96	1.00	0.98	
upper Skagit River	0.29	0.44	0.99	0.96	

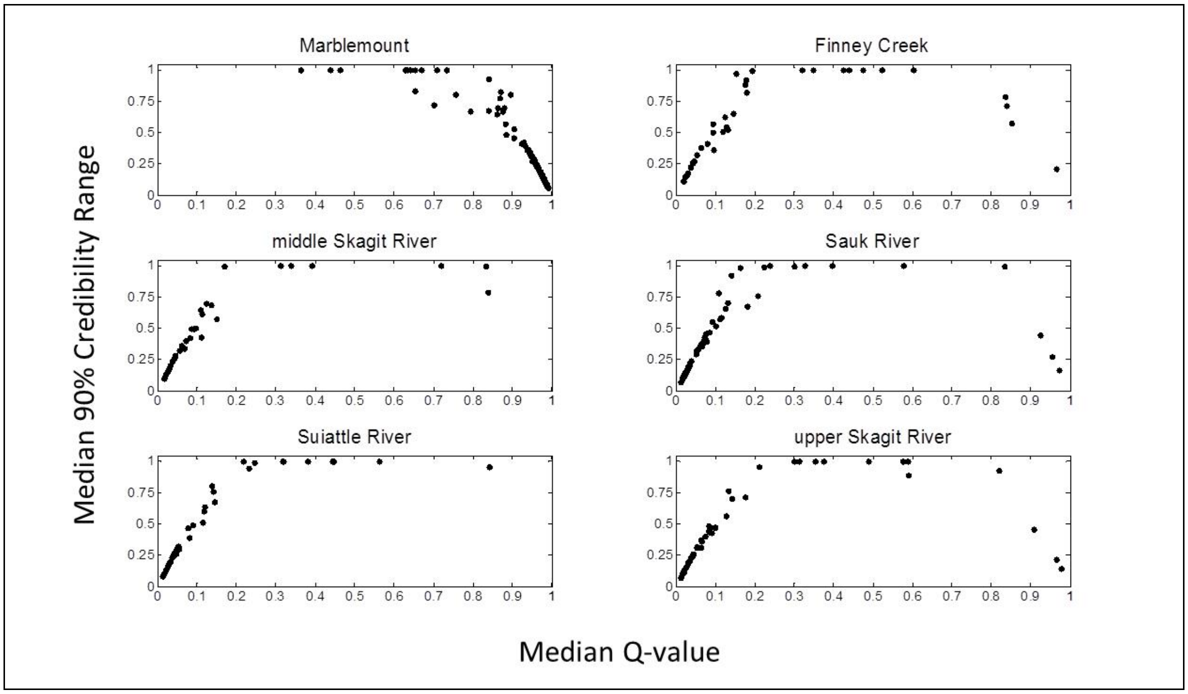


Figure 34. 90% Credibility Interval (CI) range as a function of Q-value for each adult individual from all wild and Marblemount Hatchery populations. Points represent median values across 10 STRUCTURE iterations.

Table 36. Same as Table 35, except substituting Bogachiel Hatchery for Marblemount Hatchery.

Source	Median Q-value across 10 iterations				*Total Count*
	<= 0.20	>=0.80	>0.25 and <0.75		
	Wild	Hatchery	Introgressed	Unidentified	
Bogachiel Hatchery	0.01	0.94	0.04	0.01	*136*
Finney Creek	0.73	0.02	0.17	0.07	*41*
middle Skagit River	0.91	0.00	0.06	0.03	*35*
Sauk River	0.95	0.04	0.00	0.01	*82*
Suiattle River	0.98	0.00	0.02	0.00	*44*
upper Skagit River	0.93	0.05	0.02	0.00	*55*
Total Count	*235*	*135*	*17*	*6*	*393*
Bogachiel Hatchery	0.69	0.18	1.00	1.00	
Finney Creek	0.22	0.12	1.00	0.77	
middle Skagit River	0.27	-	1.00	1.00	
Sauk River	0.17	0.42	-	1.00	
Suiattle River	0.22	-	1.00	-	
upper Skagit River	0.21	0.15	0.89	-	

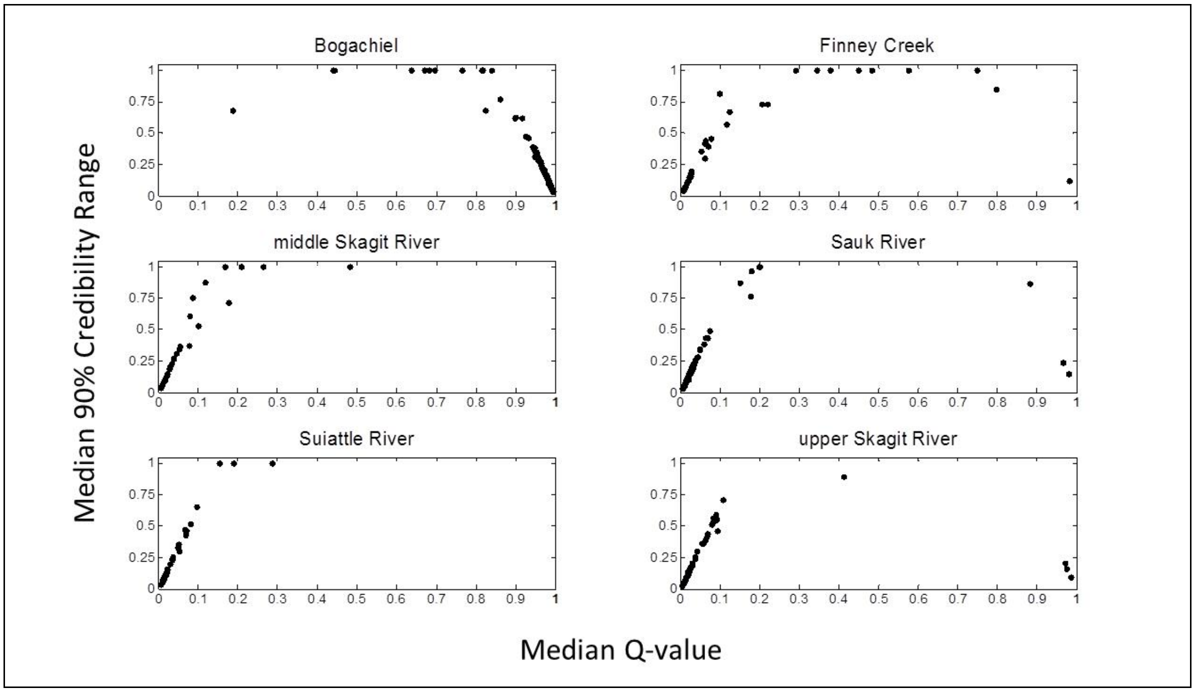


Figure 35. 90% Credibility Interval (CI) range as a function of Q-value for each adult individual from all wild and Bogachiel Hatchery populations. Points represent median values across 10 STRUCTURE iterations.

There were fewer differences in assignments between the Marblemount and Bogachiel hatchery analyses of the juvenile samples than there were for the adult samples (Tables 37 and 38, Figures 36 and 37). None of the individuals from either Bogachiel or Marblemount hatcheries were assigned to the wild group, and only few individuals were assigned as introgressed, resulting in 0.96 and 0.97 of these individuals being correctly assigned as hatchery fish, respectively. The proportions of the wild populations assigned as introgressed were more similar between the Marblemount and Bogachiel hatchery analyses for the wild juvenile fish than they were for the wild adult fish, however, there were some exceptions. The proportion of the juvenile fish from Diobsud Creek assigned as introgressed dropped from 0.15 to 0.00 between the Marblemount and Bogachiel hatchery analyses, respectively. In fact, in the Bogachiel Hatchery analysis all of the Diobsud Creek individuals assigned as wild fish. As in the analysis of the adult populations, Finney Creek showed the greatest number of juvenile individuals assigned as introgressed, although juvenile individuals from Goodell Creek, Grandy Creek and Suiattle River had a comparatively moderate number of individuals assigned as introgressed (Table 37 and 38, Figures 36 and 37). Grandy Creek had the highest proportion of individuals assigned as pure hatchery, and in the Bogachiel Hatchery analysis the number of Grandy Creek individuals assigned as pure hatchery was greater than that assigned as introgressed (Table 38). For those populations with individuals assigned as pure hatchery, that number remained unchanged between the Marblemount and Bogachiel hatchery analyses for the lower Cascade River, Grandy Creek, and Sauk and upper Skagit river populations (Tables 37 and 38). Finally, Finney Creek, and especially Grandy Creek have individuals with Q-values >= 0.80 and with relatively small 90% CI ranges (Figure 37).

Table 37. Same as Table 35, except substituting wild juvenile populations for wild adult populations.

Source	Median Q-value across 10 iterations				*Total Count*
	<= 0.20	>=0.80	>0.25 and <0.75		
	Wild	Hatchery	Introgressed	Unidentified	
Marblemount Hatchery	0.00	0.97	0.02	0.01	*117*
Bacon Creek	0.77	0.06	0.10	0.06	*48*
lower Cascade River	0.84	0.03	0.06	0.08	*79*
County Line Ponds	0.87	0.06	0.07	0.00	*54*
Diobsud Creek	0.82	0.00	0.15	0.03	*39*
Finney Creek	0.58	0.09	0.23	0.10	*91*
Goodell Creek	0.74	0.02	0.20	0.05	*61*
Grandy Creek	0.67	0.11	0.15	0.07	*61*
Sauk River	0.74	0.04	0.13	0.09	*23*
Suiattle River	0.80	0.02	0.16	0.02	*56*
upper Skagit River	0.76	0.04	0.14	0.06	*50*
Total Count	*421*	*142*	*83*	*33*	*679*
Marblemount Hatchery	-	0.26	1.00	0.97	
Bacon Creek	0.40	0.73	1.00	0.95	
lower Cascade River	0.34	0.49	0.98	0.99	
County Line Ponds	0.33	0.47	1.00	-	
Diobsud Creek	0.41	-	0.96	0.85	
Finney Creek	0.37	0.59	0.98	0.93	
Goodell Creek	0.29	0.64	0.99	0.76	
Grandy Creek	0.41	0.21	1.00	0.86	
Sauk River	0.31	0.37	0.96	0.86	
Suiattle River	0.34	0.74	0.96	1.00	
upper Skagit River	0.36	0.80	0.96	0.84	

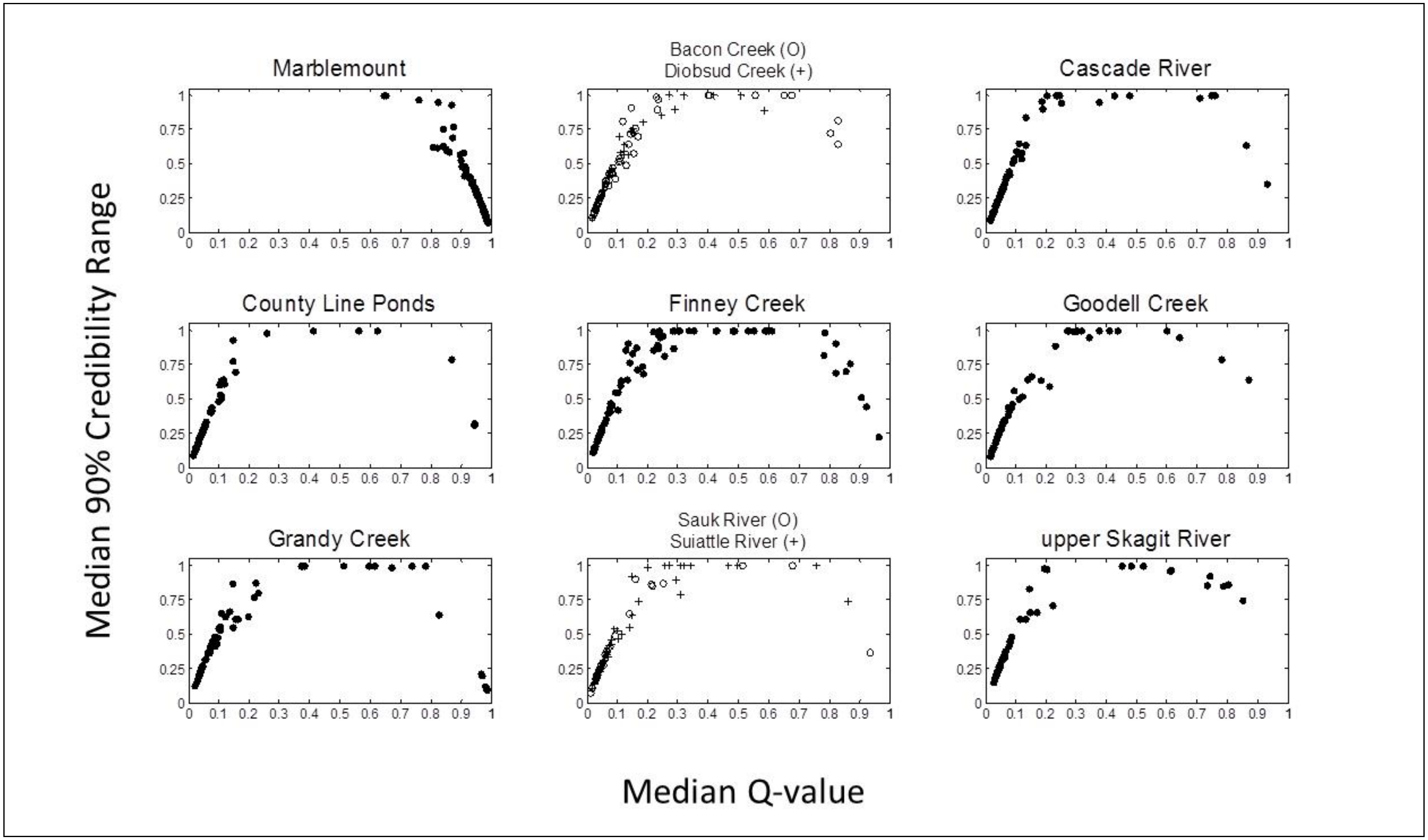


Figure 36. 90% Credibility Interval (CI) range as a function of Q-value for each juvenile individual from all wild and Marblemount Hatchery populations. Points represent median values across 10 STRUCTURE iterations.

Table 38. Same as Table 37, except substituting Bogachiel Hatchery for Marblemount Hatchery.

Source	Median Q-value across 10 iterations				*Total Count*
	<= 0.20	>=0.80	>0.25 and <0.75		
	Wild	Hatchery	Introgressed	Unidentified	
Bogachiel Hatchery	0.00	0.96	0.04	0.00	*136*
Bacon Creek	0.92	0.00	0.08	0.00	*48*
lower Cascade River	0.90	0.03	0.08	0.00	*79*
County Line Ponds	0.91	0.04	0.06	0.00	*54*
Diobsud Creek	1.00	0.00	0.00	0.00	*39*
Finney Creek	0.81	0.03	0.15	0.00	*91*
Goodell Creek	0.89	0.00	0.08	0.03	*61*
Grandy Creek	0.79	0.11	0.10	0.00	*61*
Sauk River	0.91	0.04	0.04	0.00	*23*
Suiattle River	0.88	0.00	0.09	0.04	*56*
upper Skagit River	0.86	0.04	0.04	0.06	*50*
Total Count	*492*	*147*	*52*	*7*	*698*
Bogachiel Hatchery	-	0.17	0.96	-	
Bacon Creek	0.25	-	0.97	-	
lower Cascade River	0.21	0.77	1.00	-	
County Line Ponds	0.17	0.66	1.00	-	
Diobsud Creek	0.27	-	-	-	
Finney Creek	0.31	0.61	1.00	-	
Goodell Creek	0.20	-	0.97	0.81	
Grandy Creek	0.24	0.27	1.00	-	
Sauk River	0.17	0.61	1.00	-	
Suiattle River	0.22	-	0.95	1.00	
upper Skagit River	0.22	0.85	1.00	0.94	

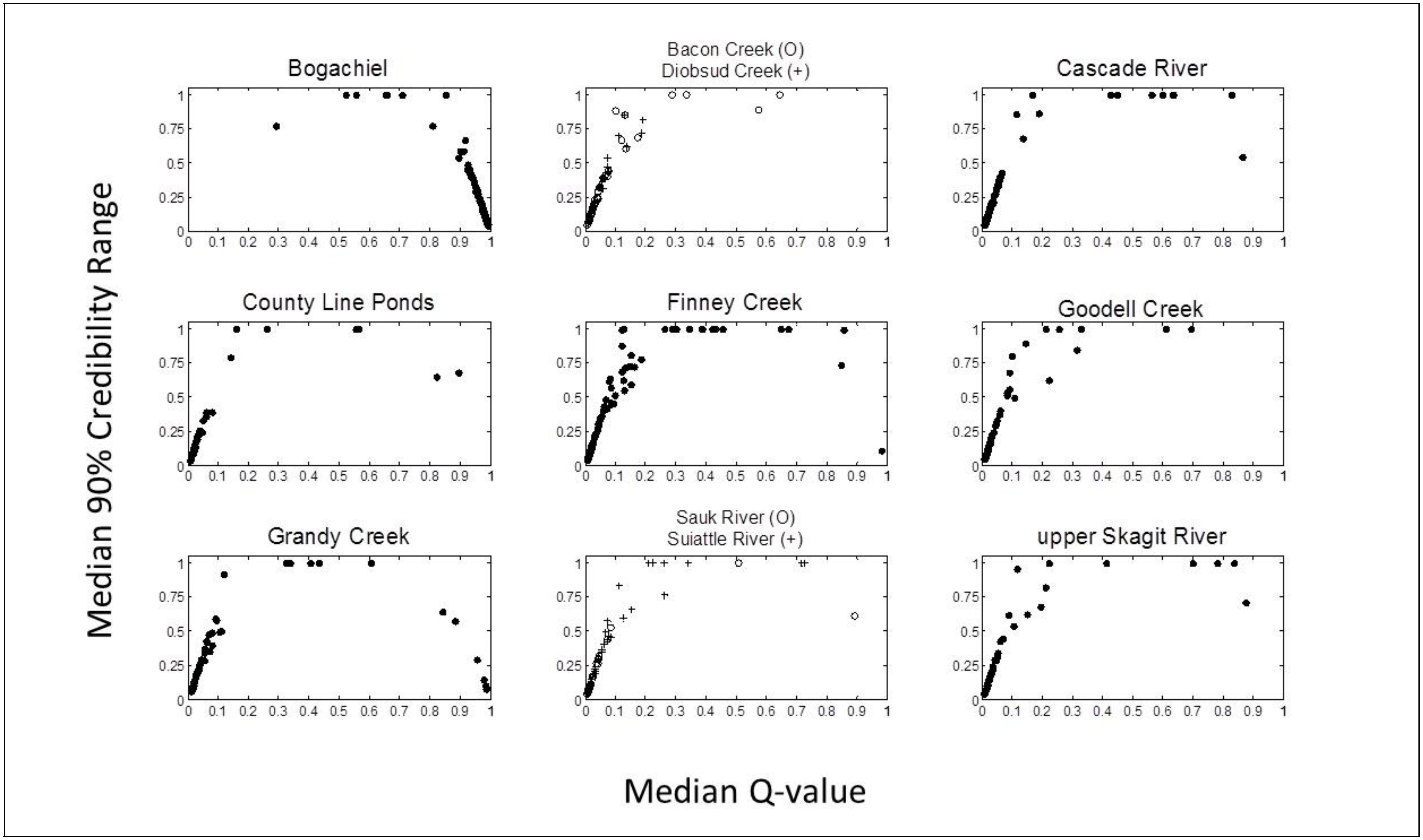


Figure 37. 90% Credibility Interval (CI) range as a function of Q-value for each juvenile individual from all wild and Bogachiel Hatchery populations. Points represent median values across 10 STRUCTURE iterations.

10.4 Discussion/Conclusions

We have no confidence to quantify introgression rate between Marblemount Hatchery (Chambers Creek) and wild Skagit River steelhead using the SPAN microsatellite data. The simulation and empirical model indicate that there would be a high assignment error rate if the SPAN microsatellites were used to assign to origin (hybrid versus pure) F1 hybrid steelhead naturally occurring within the Skagit River basin. Under conditions where hybrids were 50% of the natural population, the simulation and empirical model indicated that using STRUCTURE and the SPAN microsatellites to assign individuals as hybrid or wild would underestimate the number of hybrid fish. However, as the hybrid proportion decreased, the error rate switched from negative to positive, meaning that at lower true hybrid proportions I would overestimate the hybrid proportion, and this overestimation could be as high as 200-600%. We do not know *a priori* the hybrid proportion of the unmarked populations (i.e., we do not have an *a priori* expectation of the error rate). Whatever hybrid proportion I calculate using the SPAN microsatellites, that proportion may be under-representing or over-representing the total number of hybrids.

Part of the reason for the inability to clearly distinguish hybrid from pure fish lies in the fact that wild Skagit River steelhead and Chambers Creek origin steelhead (regardless of the hatchery where they are propagated) share a recent common ancestor and are currently only weakly (but significantly) differentiated ($F_{ST} \approx 0.02$, Table 19; Kassler and Warheit 2012). The SPAN microsatellite loci (and simulation loci) provide about 225 alleles (roughly 15 alleles per locus across 15 loci; Table 30). This number of alleles is clearly insufficient to quantify introgression when parent populations are differentiated by $F_{ST} = 0.02$ or lower (see also Vähä and Primmer 2006). Increasing the number of alleles (e.g. to 1,500 or 7,500) would substantially improve our ability to confidently distinguish F1 hybrids from pure hatchery or wild individuals. The 100 and 500 loci used in simulations were randomly selected with respect to their power to differentiate the hatchery and wild populations. Thus, it is conceivable that error rates can be reduced to near zero using fewer than 500 loci if loci are deliberately chosen for their power to differentiate hatchery and wild populations. It must also be noted that this analysis evaluated only the power to distinguish F1 hybrids. Substantially larger numbers of loci (alleles) most likely would be necessary to confidently distinguish F2 or backcrossed hybrids from F1 hybrids and pure wild and hatchery individuals. Introgressive hybridization will likely involve hybrid generations other than F1, but the simulation and empirical model considered only one generation of hybridization.

Although the SPAN microsatellite data provided little confidence in quantifying introgression, can the data be used in a comparative sense? That is, can we compare the reported introgression rates (i.e., proportion of population with individuals' Q-values between 0.25 and 0.75) among the wild Skagit River populations to determine if any population or set of populations are more introgressed than other populations? The 90% CI range is large and the same for pure wild, pure hatchery, and F1 hybrid fish assigned as hybrids. Therefore, I cannot differentiate between a correctly assigned hybrid and an incorrectly assigned pure wild or hatchery fish. Furthermore, since there is overlap between the 90% CI ranges of correctly assigned wild fish and F1 hybrids incorrectly assigned as wild (Q-values < = 0.20), I cannot differentiate with statistical confidence correctly assigned pure wild fish and incorrectly assigned hybrid fish. However, since it is safe to assume that all wild pure Skagit populations are more closely related to each other than any are to any Chambers Creek population (e.g., Marblemount or Bogachiel hatcheries), the assignment error associated with recency of common ancestry is most likely the same for all wild pure Skagit River populations. That is, differentiation between each of the pure populations and the Marblemount Hatchery population should be roughly the same. I can then assume that the rate of assigning pure wild fish as hybrids is the same for all populations. Following these assumptions, differences in the proportion of each population assigned as hybrid would reflect *qualitative* differences in hatchery-wild introgression among these populations. Therefore, I assume that the proportion of all individuals from a population assigned as hybrids is an "introgression signal;" populations with greater proportions assigned as hybrids will be more introgressed than populations with lower proportions assigned as hybrids.

For adult populations, Finney Creek stands out as having the strongest introgression signal among the five populations. This is particularly evident in the Bogachiel Hatchery analysis, where the number of individuals assigned as hybrids in Finney Creek greatly outnumbers that in the other four populations. The assignments rates for the wild juvenile populations generally reflect the same patterns as those for the wild adults: (1) higher levels of introgression in the Marblemount Hatchery analysis than in the Bogachiel Hatchery analysis; and (2) higher levels of introgression for the Finney Creek population than the other populations. However, unlike the adult populations, Finney Creek is not alone in showing elevated levels of introgression in juvenile fish. In fact, for both Bogachiel and Marblemount hatchery analyses, all populations (except the Diobsud Creek population in the Bogachiel Hatchery analysis) showed qualitative evidence of introgressed fish. Since I see no reason to assume that there is a higher proportion of pure wild juveniles that will be assigned as hybrids than that for pure wild adults, higher levels of introgression in juvenile populations compared with adult populations suggests that either hybrid adults are more difficult to find than pure adults due to possible temporal or spatial sorting, or juvenile to adult survival of hybrid fish may be lower than that of pure fish. However, Finney Creek is an exception in that the introgression signal is the same for adult and juvenile fish.

It is conceivable and perhaps likely that pure unmarked hatchery-origin fish and fish with pure hatchery ancestry (e.g., offspring of naturally occurring hatchery x hatchery crosses) occur on or near natural spawning areas. This means that unmarked fish assigned as pure hatchery (i.e., Q-value >= 0.80) may indeed be pure hatchery or hatchery-ancestry, and not an introgressed fish with high Q-values. However, in the simulation dataset the Q-value – 90% CI range joint distributions for correctly assigned hatchery fish and F1 hybrids incorrectly assigned as hatchery fish (Q-values >= 0.80) are nearly identical. Therefore, I do not have statistical support to differentiate pure hatchery and hybrid fish with Q-values > = 0.80, or to identify these fish as either pure hatchery or hybrid fish. That being said, Finney Creek, Sauk River, and upper Skagit River, from the adult populations, and Finney Creek and especially Grandy Creek, from the juvenile populations show Q-value – 90% CI range joint distribution patterns more extreme than what you may expect from introgressed fish (i.e., very high Q-values and low 90% CI ranges). This suggests that based on these samples pure unmarked hatchery-origin fish or fish with pure hatchery ancestry occur at a greater frequency within these creeks and rivers than other areas within the Skagit Basin.

Marblemount Hatchery currently releases smolts from the hatchery itself (Cascade River) and at the Baker River trap, downriver from Marblemount Hatchery near the town of Concrete (Figure 23) (Washington Department of Fish and Wildlife 2012). However, historically, smolts of

Chambers Creek origin (either from Marblemount Hatchery or Barnaby Slough – downriver from Marblemount Hatchery between the Sauk and Cascade Rivers) were released throughout the middle and upper Skagit River, most notably the mainstem Skagit River, Cascade River, and Grandy Creek. The confluence of the Skagit River and Finney Creek is nearly adjacent to Grandy Creek, and the middle Skagit River adult collection, and the Finney Creek adult and juvenile populations are sandwiched between the Grandy Creek and Baker River trap smolt release sites. The Finney Creek populations stand out as having a high introgression signal, and pure unmarked hatchery or hatchery-ancestry fish are suggested in the Grandy Creek, Finney Creek (adult and juvenile), and to a lesser extent the Sauk (adult) and lower Cascade river populations. Some of these populations are close to historical or current hatchery smolt release sites, but considering the introgression signal in all populations, proximity to these release sites alone is not a sufficient predictor of hatchery introgression in the Skagit River. Finally, both Finney and Grandy creeks have natural-origin steelhead spawning earlier than elsewhere in the Skagit River basin, during a time more consistent with the early-spawning Marblemount Hatchery populations (Brett Barkdull, WDFW pers. Comm, through Dave Pflug, SCL 2013). The higher introgression signal and the possible presence of pure unmarked hatchery or hatchery-ancestry fish in these two creeks are consistent with the early-spawning behavior.

10.4.1 Conclusions

The SPAN microsatellite loci lack sufficient power to reliably *quantify* Marblemount Hatchery (Chambers Creek-origin) introgression into the wild Skagit River winter steelhead populations, or reliably identify pure unmarked hatchery or hatchery-ancestry fish using the program STRUCTURE. However, under some reasonable assumptions, the Finney Creek adult and juvenile populations appeared to have a higher level of hatchery-wild introgression than all other wild populations. A few individuals from Finney Creek and especially Grandy Creek had Q-value – 90% CI range joint distribution patterns more extreme than what you may expect from introgressed fish, suggesting that these fish may be pure hatchery or hatchery-ancestry fish. Finney and Grandy creeks are proximate to or are the location of historical hatchery smolt release sites, and show an unusually early spawning period compared with other wild populations in the Skagit Basin, but similar to the Marblemount Hatchery populations. These data suggest that there are pockets of winter steelhead spawning areas within the Skagit Basin where pure hatchery or hatchery-ancestry fish exist and have hybridized with native steelhead fish.

Increasing the number of loci genotyped for each individual decreased the assignment error rates, to the point where there would be, effectively, no error in assigning F1 hybrid individuals. Thus, I recommend that single nucleotide polymorphism (SNP) loci be developed specifically for Skagit River and Marblemount Hatchery steelhead and be used to quantify hatchery introgression among the steelhead populations within the Skagit River basin. In addition, I am currently working with a colleague on a statistical approach that would provide a posterior probability density function for the identity (pure wild, pure hatchery, or hybrid) of each fish given the joint distribution of Q-values and 90% credibility ranges from the simulated data, as in Figure 5. This would enable us to assign a fish to a category (pure versus hybrid, for example) using an explicit statistical test (e.g., log likelihood ratios) or by the strength of posterior probabilities, or to establish empirically-based Q-value thresholds that would define pure hatchery, pure wild, and F1 hybrids

11.0 Effects of Hatchery Smolt Release Practices and Environmental Factors on Native Skagit Steelhead Populations

11.1 Introduction

The abundance of wild and native steelhead in the Skagit River has undergone a major decline since the mid 1980s, and frequently fell below the escapement goal floor level of 6,000 spawners during the 2000s. Steelhead in the Skagit River, along with other populations in the Puget Sound, were listed as “threatened” under the Endangered Species Act in 2007 as a result of major declines in abundance and productivity observed during the 1990s and 2000s. Steelhead escapement in the Skagit has remained low throughout the 2000s even though recreational and tribal harvest has been largely curtailed since the mid-1990s. The abundance of Skagit steelhead currently is a small fraction of its historic run size estimates and current intrinsic potential.

Historic Skagit River steelhead run size estimates that were based on a Bayesian analysis of commercial catch records at the turn of the twentieth century (1895) ranged between 70,000–149,000 steelhead with a mean of 105,600 and a mode of 86,700 steelhead (Gayeski et al. 2011). This estimate is ten times greater than present run-sizes from 1980 to 2004 (Gayeski et al. 2011). This was not all explained by the 33% loss of stream length available in Puget Sound, nor the indices of ocean conditions leading up to the 1895 steelhead return, such as the Pacific Decadal Oscillation (PDO), which was shown to have been unfavorable during that time period (Gayeski et al. 2011). Comparatively, the present escapement goal (or “escapement floor” as more recently termed) of 6,000 wild steelhead (Appendix 1) represents 4-8 percent of these historic run-size estimates. The Skagit River steelhead escapement level has been met six times in the 14 year period between 1998 and 2011 (UST-a). The decrease in abundance post 1985 was also identified in documents published during that time which stated that steelhead in the Skagit were overfished by 1920, and populations were only a portion of what they had been in the late 1800s (Smith and Anderson 1921). The capacity for the Skagit River to produce more steelhead is also evident from recent habitat-based capacity estimates. The Puget Sound Steelhead Technical Recovery Team (TRT) used a habitat based (IP) approach and estimated wild steelhead capacity of ~ 54,000 for the Mainstem Skagit Historical Demographically Independent Population (DIP), ~4,300 for the Baker River DIP, and over 18,000 for the Sauk River DIP. The resulting total was 78,068 historic wild Skagit basin steelhead capacity was estimated to be over 76,000 (Hard et al. 2012), far greater than the 6,000 escapement goal.

The objective of this study was to identify the potential long-term impacts of hatchery programs, with respect to other factors, on steelhead returns in the Skagit River. The Skagit River is only second to the Snohomish River in the number of non-native hatchery steelhead smolts that have been released (annual average of 346,000 for Skagit and 476,000 for Snohomish) into a watershed over the past 50 years in the Puget Sound (Hard et al. 2007; WDFW SASI database 2008). The large majority (92%) of Skagit smolt releases have been

Chambers Creek origin fish. The annual number of hatchery releases has substantially increased since the mid-1980s (average annual increase of 2.6%). The abundance of native Skagit steelhead spawners had declined over this same period (average annual decline of 3.6%). Hatchery smolt release programs have been found to have genetic and ecological impacts on native steelhead populations that negatively effect their long-term abundance and productivity (Chilcote 2003; Kostow and Zhou 2006; Kostow 2009).

In addition to non-native hatchery smolt releases, a number of environmental factors have been identified that could be responsible for the decline in native steelhead populations in the Puget Sound that has been observed since the mid 1980s, including changes in climate (shifts in coastal upwelling and ocean conditions, snowpack, and streamflow), and harvest management (Hard et al. 2007; Ford 2011), and continuing degradation of freshwater, estuary, and nearshore habitat (Scott and Gill 2008). There is growing evidence that the steep decline in the abundance of native steelhead populations in the Puget Sound have been caused to a major extent by poor ocean conditions that have occurred since the 1980s. Many of the steelhead populations in the Puget Sound, the Georgia Basin, and the northeast coast of Vancouver Island have undergone a similar pattern of decline during this period (Hard et. al. 2007; Ward 2000; Welch et. al 2000), suggesting that declining marine survival rates are impacting these populations. Hatchery steelhead stocks in the Puget Sound have also undergone a major decline during this same period, with average smolt-to-adult return (SAR) rates declining from 7.0% in the mid 1980s to 0.2% in the 2000s (Scott and Gill 2008). There is also evidence that the frequency of peak flows has been increasing in the Skagit watershed, particularly in the Sauk River drainage (USGS gaging data). Peak flows have been identified as a major factor negatively impacting the freshwater survival of Chinook salmon in the Skagit River watershed (Kinsel et. al 2008; Zimmerman et al. *In Prep*). Freshwater survival rates for steelhead would be expected to be negatively impacted by peak flows as well, especially since steelhead have a much longer juvenile residency period in freshwater compared to Chinook and are therefore potentially more vulnerable to peak flow impacts.

In order to better understand how long-term releases of non-native hatchery smolts potentially impact native steelhead populations in the Skagit, we first examined the individual effects of hatchery smolt releases, ocean conditions, and peak flows on native steelhead returns using a linear regression analysis. We also calculated changes in freshwater survival trends (egg-to-smolt-survival) and marine survival trends (SARs) during this period to identify if the declines in native steelhead returns in the Skagit were related to changes in freshwater survival, marine survival, or both. We then completed a multiple regression analysis that examined the combined influence of three independent variables - Skagit hatchery smolt release numbers, surface sea temperatures in the North Pacific, and peak flows in the Skagit – on native Skagit steelhead adult returns. This analysis also allowed us to identify the long-term effects of hatchery smolt releases on native steelhead returns while controlling for the influence of the other two independent variables. Next, we conducted an analysis of the effects of non-native hatchery smolt releases on native steelhead productivity among major river basins in the Puget Sound. This was done to provide regional corroboration for our hypothesis that releases of non-native hatchery fish have had a long-term impact on native steelhead populations in the Skagit River basin. Finally, we conducted a literature review to provide evidence from other regions in the Pacific Northwest that hatchery releases have a negative and long-term impact on native steelhead populations.

11.2 Methods

We employed a multiple-step approach for analyzing the effect of hatchery smolt releases, ocean conditions, and streamflow conditions on native Skagit steelhead populations. First we conducted a descriptive analysis to examine correlations and trends in each of the factors that can affect the return of native Skagit steelhead. Linear regression analyses were conducted in some circumstances to assess the relative influence of a number of key environmental factors on wild steelhead returns in the Skagit. As part of the descriptive analyses, we also calculated changes in freshwater survival trends (egg-to-smolt-survival) and marine survival trends (smolt-to-adult return survival) between 1993 and 2007 to identify if the declines in native steelhead returns in the Skagit could be due to changes in freshwater or marine survival.

11.2.1 Freshwater Survival

To assess recent trends in the freshwater productivity of wild steelhead in the Skagit River, we calculated egg-to-smolt survival rates from 1993 to 2007. Annual egg production was estimated from spawner escapement data for the Skagit (WDFW SASI database 2011), which was then multiplied by sex ratio and fecundity estimates for Skagit steelhead. Smolt outmigration estimates were calculated using WDFW smolt trap wild steelhead catch values (Kinsel et al. 2008) divided by a trap efficiency estimate obtained from known annual releases of hatchery smolts. Based upon scale-aging data for adult steelhead scales collected in the Skagit, we assumed that 1% of steelhead smolts outmigrated at Age 1, 74% outmigrated at Age 2, 24% outmigrated as Age 3, and 1% outmigrated as Age 4 (note: the ages are based upon the presence of annuli on scales, which are typically formed during the early winter). The WDFW smolt trap on the Skagit River has not yet been calibrated using efficiency estimates from wild steelhead juveniles, so the estimates used should regarded as a relative estimate (or index) of egg-to-smolt survival.

11.2.2 Ocean Survival

Trends in marine survival rates are best described by the smolt-to-adult return (SAR) rate, which is calculated by dividing the number of adults returns (pre-harvest estimate) by the number of smolts outmigrant that produced these returns. We calculated SAR rates for both hatchery and wild steelhead stocks from the Skagit River. Hatchery smolt release numbers were obtained from WDFW records for the Marblemount Hatchery (WDFW 2011). Smolt outmigrant numbers for wild steelhead were calculated using data from the WDFW smolt trap located on the lower Skagit River, employing methods described in the previous section. Adult return numbers for hatchery and wild steelhead from the Skagit were obtained from WDFW estimates (WDFW 2011), which are based on hatchery returns or wild escapement numbers, and ocean and terminal area harvest estimates.

11.2.3 Linear Regression Analysis

We employed a linear regression analysis to examine the relationship of three independent variables – Skagit hatchery smolt release numbers, surface sea temperatures, and Skagit peak flows – on the annual abundance of returning adult steelhead to the Skagit River. Annual values for native steelhead escapement and returns, and annual hatchery smolt release numbers, were obtained from the WDFW Steelhead Historical Database (http://wdfw.wa.gov/publications/00150). To examine the potential impacts of hatchery releases on wild steelhead returns, we calculated the cumulative number of hatchery smolts released into the river over a period of one, two, three, and four wild steelhead generations (i.e., 5, 10, 15, and 20 years). This was done by calculating a moving total hatchery release number on an annual basis determined from the preceding 5, 10, 15, or 20 years. We then calculated the goodness-of-fit (R-Square value) for these four hatchery release measures with wild steelhead return numbers for the Skagit. We found that the cumulative number of hatchery smolts released over a 15-year period explained the greatest amount of variability in wild steelhead returns, since the goodness-of-fit value was highest for this smolt release variable. We then completed a linear least-squares regression relationship to determine the effect of hatchery releases over the preceding 15 years on wild steelhead returns in the Skagit.

We calculated mean annual values of the Pacific Decadal Oscillation Index (PDO), which is a measure of surface sea temperatures in the North Pacific that has been found to be significantly correlated with the marine productivity of salmon (Mantua et al. 1997), from historical monthly data published by the University of Washington Climate Impacts Group (http://jisao.washington.edu/pdo/PDO.latest). The PDO index ranges in value from -3.6 to +3.6, with 0.0 representing average surface sea temperatures from the historical record, values less than zero representing cooler than average values, and values greater than zero representing warmer than average values (Hare and Mantua 2000). Annual peak flow values for the Skagit River were obtained for the Mt Vernon gaging station (USGS gage 12200500) from the USGS peak streamflow database for the State of Washington (http://nwis.waterdata.usgs.gov/wa/nwis/peak). We calculated a least-squares fit regression between each independent variable and native steelhead returns using the SYSTAT 12 statistics package (Systat Software Inc. 2007).

11.2.4 Multiple Regression Model

We completed a multiple regression model on the combined effects of a number of independent variables, including hatchery smolt release numbers, on wild steelhead returns in the Skagit (the dependent variable). This was done to control for possible intercorrelations between hatchery release numbers and variables related to shifting ocean conditions and hydrological conditions. We included a number of independent variables, including annual hatchery release numbers (15-year moving total), the PDO index, annual peak flows, annual low flows, and mean annual flows, in this analysis. We then eliminated those variables that did not explain sufficient variability for the independent variable, or which were highly correlated with other independent variables. The combined influence of these three independent variables on native Skagit steelhead adult returns was then determined using a least-squared multiple regression analysis using the SYSTAT 12 statistics package. Multicollinearity among the independent variables was assessed using the “tolerance” metric (Draper and Smith 1998).

11.2.5 Regional Analysis of Hatchery Smolt Release Effects on Steelhead Productivity

We conducted a regional analysis on the effects of smolt releases on the productivity of wild steelhead productivity among a number of major Puget Sound river basins. This was done to determine if there is a large-scale pattern between hatchery releases and wild steelhead population trends in the region. If a regional pattern in hatchery effects is found to be present, then it would provide further support for the concern that hatchery practices have impacted wild steelhead populations in the Skagit River. The regional analysis was conducted by using wild steelhead escapement numbers. Long-term steelhead spawner escapement data is available for a number of Puget Sound watersheds, though this list is fairly short compared to the number of watershed where steelhead populations are present. Escapement data for these watersheds were acquired from the WDFW Puget Sound Steelhead Historic Database (2011). The watershed we included in our analysis include six in the northern Puget Sound (Samish, Skagit, Stillaguamish, Skykomish, Snoqualmie, and Tolt rivers), three in the central and southern Puget Sound (Green, Puyallup, and Nisqually rivers), and two in the Hood Canal (Skokomish and Tahuya rivers).

We calculated the mean density of wild spawning steelhead in each Puget Sound river basin over the past five years (2006-2001) by dividing the mean escapement values by the total watershed area that is accessible to spawning in each watershed. The watershed area data was derived from the NOAA steelhead GIS database (Ford 2011). We then compiled data on the annual number of hatchery-produced steelhead smolts released into these rivers from several sources, including the WDFW Puget Sound Steelhead Historic Database (2011), and data published by NOAA (Hard et. al 2008). The average annual number of winter stock and summer stock hatchery smolts released into each system were then calculated from this data for each watershed. We also calculated the average number of steelhead hatchery smolts released into each of these watersheds in relation to watershed area. This provided an estimate of the relative density of hatchery smolts released into each watershed on an annual basis.

Finally, we calculated the intrinsic population growth rate for wild steelhead populations in these watersheds from 1985 to the 2010. Steelhead populations in most Puget Sound watersheds peaked in around 1985, and subsequently declined to current low levels throughout most of the 1990s and 2000s. The mean annual population growth rate (lambda) was calculated for each watershed from WDFW escapement data for this 27-year period. Average growth rates (lambda values) were calculated using an exponential growth function in Microsoft Excel. We then determined the effect that historic hatchery smolt releases had on wild steelhead growth rates using a least-squares fit linear regression analysis. We used the average number of hatchery smolts released per wild spawner as the driving (independent) variable for this analysis. This metric is not substantially influenced by watershed area, so it can be used to evaluate hatchery effects on wild steelhead populations among watersheds of different sizes. The average annual number of hatchery steelhead smolts released within each watershed over the 15-period prior to 2011 was calculated from WDFW data, and was then divided by the annual average steelhead escapement for this same period. The resulting ratio variable (hatchery smolts released per spawner) was used to predict wild steelhead population growth among the Puget Sound watersheds.

11.3 Results

11.3.1 Annual Returns of Native Steelhead to the Skagit River

Returns of native steelhead spawners to the Skagit River basin increased rapidly from late 1970s (the period when WDFW and the Puget Sound treaty tribes first started counting native and wild steelhead separately from hatchery returns) to the mid-1980s, reaching peak values of almost 16,000 in 1988 (Figure 38). During the early 1990s, numbers of returning steelhead substantially declined to less than 8,000 fish per year. As a result of declining returns, recreational and tribal harvest was reduced in the Puget Sound by the fisheries co-managers (WDFW and treaty tribes). Returns of steelhead further declined in the 2000s, even though recreational and tribal harvest for native steelhead was reduced to historically low levels through fishing closures. The average annual decline in Skagit River native steelhead returns from 1988 through 2010 was 3.6%. As a result of the historically low numbers of steelhead observed during this period, Puget Sound steelhead were listed as threatened under the Endangered Species Act by NOAA Fisheries in 2007.

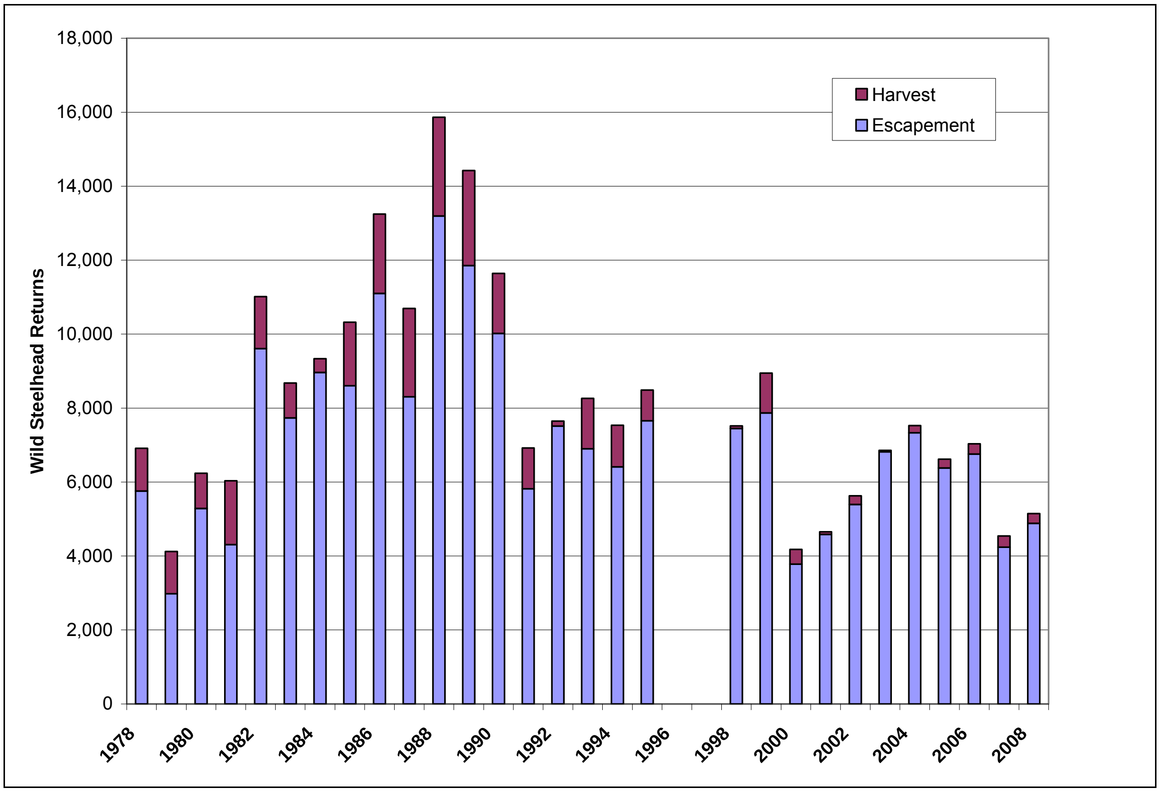


Figure 38. Annual abundance (escapement plus total harvest) of wild steelhead adult returning to the Skagit River basin, 1978 – 2008.

11.3.2 Trends in Hatchery Smolt Releases Since 1960

The Skagit River has been the location of the one of the largest non-native hatchery smolt release programs for steelhead in the Puget Sound. This program, managed by the WDFW in coordination with Treaty Tribes, was developed to provide a non-native steelhead fishery for recreational and tribal harvest. The steelhead hatchery program on the Skagit has historically involved several donor stocks, the most numerous being a winter-run steelhead that originated from Chambers Creek, Washington. From 1960 through 2010, Chambers Creek origin fish have accounted for 92% of hatchery smolts released in the Skagit River basin. Most of these fish were raised at the State of Washington's Marblemount Fish Hatchery, which is located on the Cascade River in the upper Skagit River basin. During the early 1960s, releases of winter-run hatchery smolts numbered around 100,000 fish per year (Figure 39). By the mid-1960s, releases of winter-run hatchery smolts on the Skagit varied between 200,000 and 550,000 fish per year, with released in most years up to the early 1990s between 200,000 and 300,000 winter-run smolts.

From 1970 through 1980, the State of Washington released significant numbers of summer-run hatchery steelhead in the Skagit River basin (Figure 39). Most of these summer-run fish originated from the Skamania hatchery on the Columbia River. Annual releases on summer-run smolts during this 10-year period ranged from approximately 20,000 to 100,000 fish. Smaller numbers of summer-run hatchery smolts were released into the Skagit River basin during the 1980s and 1990s, with annual releases totaling less than 20,000 fish per year. The release of summer-run hatchery fish was discontinued in the Skagit in the late 1990s.

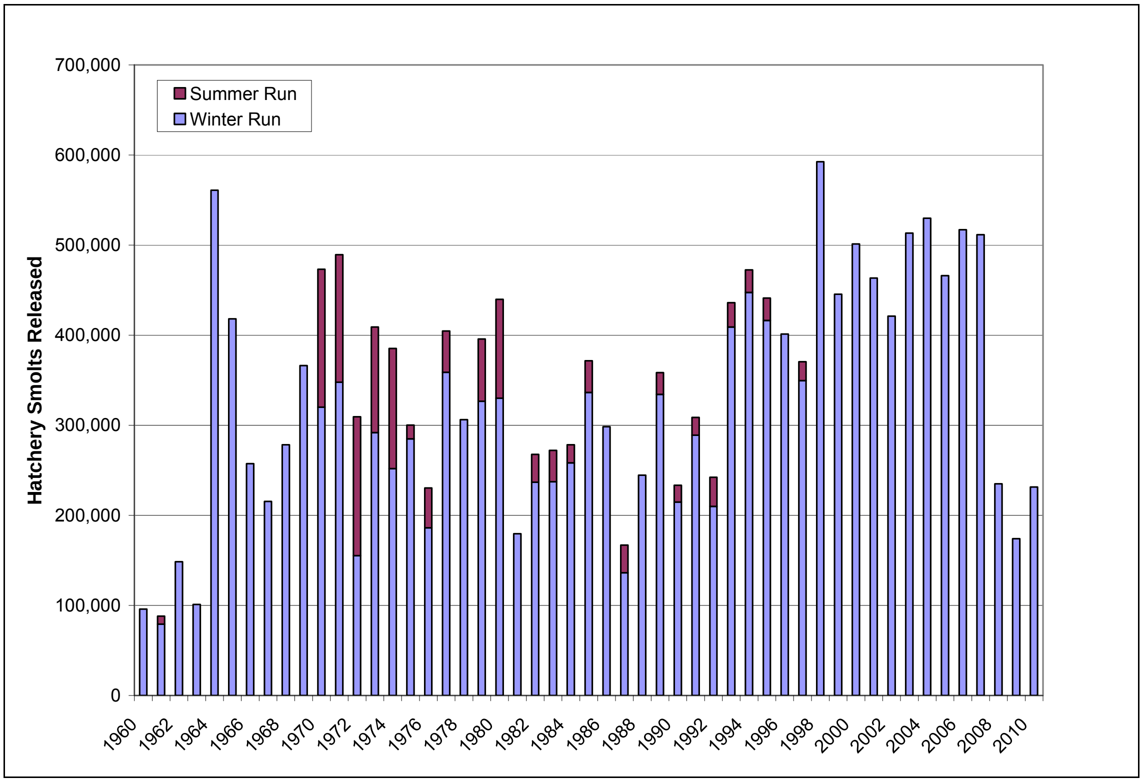


Figure 39. Mean annual release numbers for winter run and summer run hatchery smolts in the Skagit River basin, 1960 – 2010.

In response to several years of declining hatchery adult steelhead returns, the number of winter-run hatchery smolts released into the Skagit River was increased in the mid-1990s (Figure 39). Starting in 1994 and continuing through 2007, annual releases of winter-run hatchery smolts ranged from 350,000 to almost 600,000 fish, and exceeded 400,000 fish in most years. Starting in 2008, annual releases of hatchery winter-run steelhead were reduced to less than half of the number released during the early and mid 2000s. Since 2008, releases of winter-run hatchery smolts have ranged from about 180,000 to 230,000 fish per year.

The practice of releasing increasingly larger numbers of hatchery smolts during a period when wild returns were declining resulted in an inverse relationship between hatchery smolt releases and adult returns (Figure 40). As a result of this practice, the annual number of hatchery releases has increased (average annual increase of 2.6%) during a period when abundance of native Skagit steelhead spawners had declined (average annual decline of 3.6%).

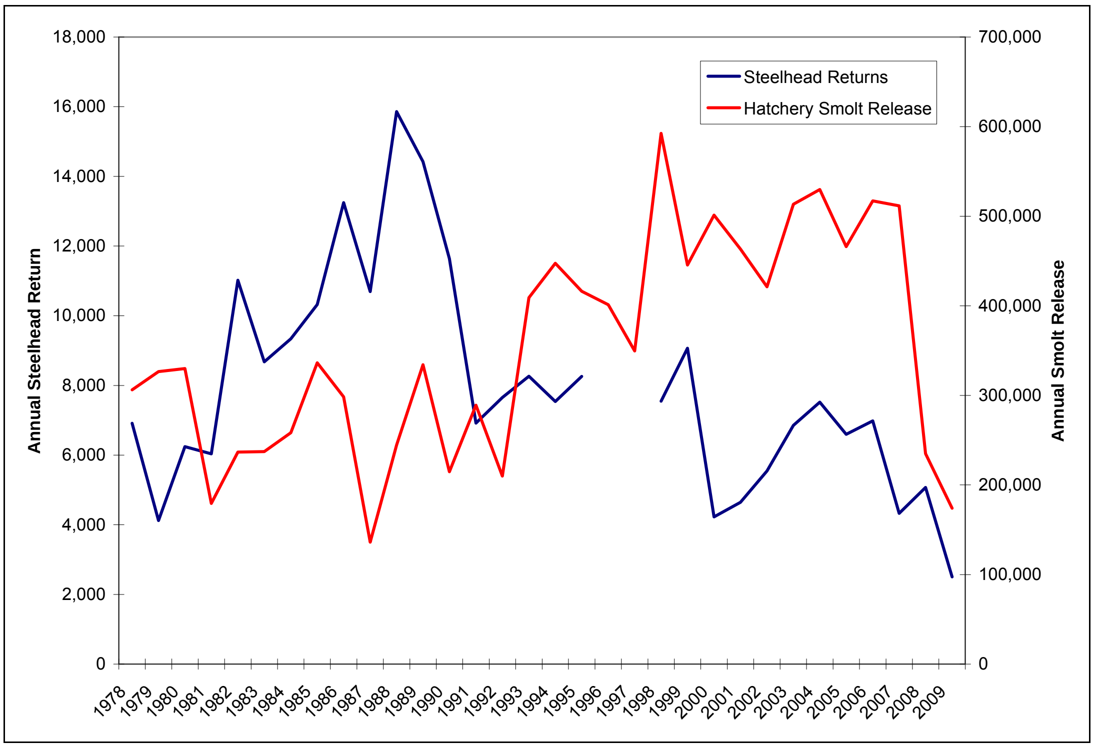


Figure 40. Inverse relationship between cumulative hatchery smolt releases (moving total for 15-year period) and wild steelhead returns for the Skagit River, 1978 – 2010.

11.3.3 Shifts in Ocean Conditions

Shifts in ocean conditions have been identified as a major factor affecting the abundance of salmon in the Pacific Northwest (Mantua et al. 1997; Hare and Mantua 2000). Populations of Chinook salmon, coho salmon, and other north Pacific fish species have been found to be positively correlated to extended periods of cold surface water temperatures in the north Pacific. Periodic cycles in surface sea temperatures in the north Pacific can be measured using the Pacific Decadal Oscillation (PDO) index. The PDO Index was neutral (near zero) in the late 1970s, and then shifted to positive values (indicating warmer surface temperatures) in the early 1980s, with the greatest index values (warmest temperatures) observed from 1984 through 1988 (Figure 41).

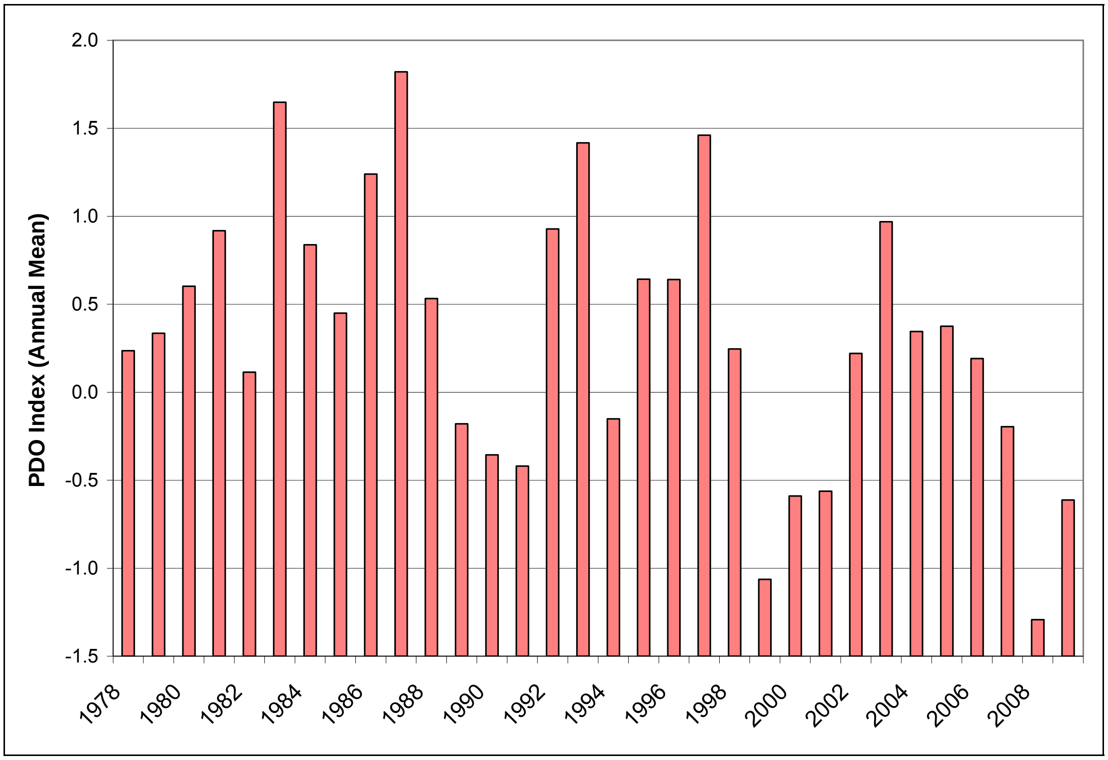


Figure 41. Annual mean values of the Pacific Decadal Oscillation (PDO) Index; 1978 – 2009. The PDO Index is a measure of surface sea temperatures in the North Pacific Ocean (source: Univ. of Washington Climate Impacts Group 2012).

The PDO Index shifted to negative values in the early 1990s, meaning that sea temperatures in the north Pacific had become substantially cooler. The PDO Index increased to positive values again by the mid 1990s, which reflected a shift to warmer sea temperatures again. The PDO index fell to sharply negative values in the early 2000s, with values either neutral or negative throughout much of the 2000 decade (with exception to 2003). This means that water temperatures in the north Pacific have been substantially cooler during the 2000s when compared to the warmer regime observed in the mid 1990s, and the even warmer regime observed in the mid 1980s.

11.3.4 Trends in Freshwater Conditions

High flow events (peak flows) are another factor likely impacting the number of steelhead returning to the Skagit River. Peak flows impact several life stages of steelhead, including spawning, egg and embryo incubation, and juvenile rearing, and are thus a key factor to the freshwater productivity of Skagit populations. Peak flows that exceed bankfull conditions, which have a return-interval of approximately 2 years (Dunne and Leopold 1978), result in bedload movement and sediment scour that can substantially reduce the survival eggs and embryos incubating within gravels and cobbles in river and stream channels. Peak flows that occur during the spawning period of steelhead in the Skagit River (March through June) can result in spawning along the margins of the river channel, which increases the susceptibility of redds to dewatering when flows decline during the late spring and summer. Peak flows can impact rearing juvenile steelhead by displacing individuals from their natal rearing areas, and by causing physical injury and mortality due to substrate movement and sediment scour.

Assuming that survival rates of juvenile steelhead are reduced when flows exceed 80,000 cfs at the Mt Vernon flow gaging station, then low survival rates would have been expected due to peak flows that occurred in 8 of the past 32 years (Figure 42). The most severe flood events on the Skagit over this period occurred during the 1981, 1982, 1990, 1991, 1996, 2004, and 2007 water years.

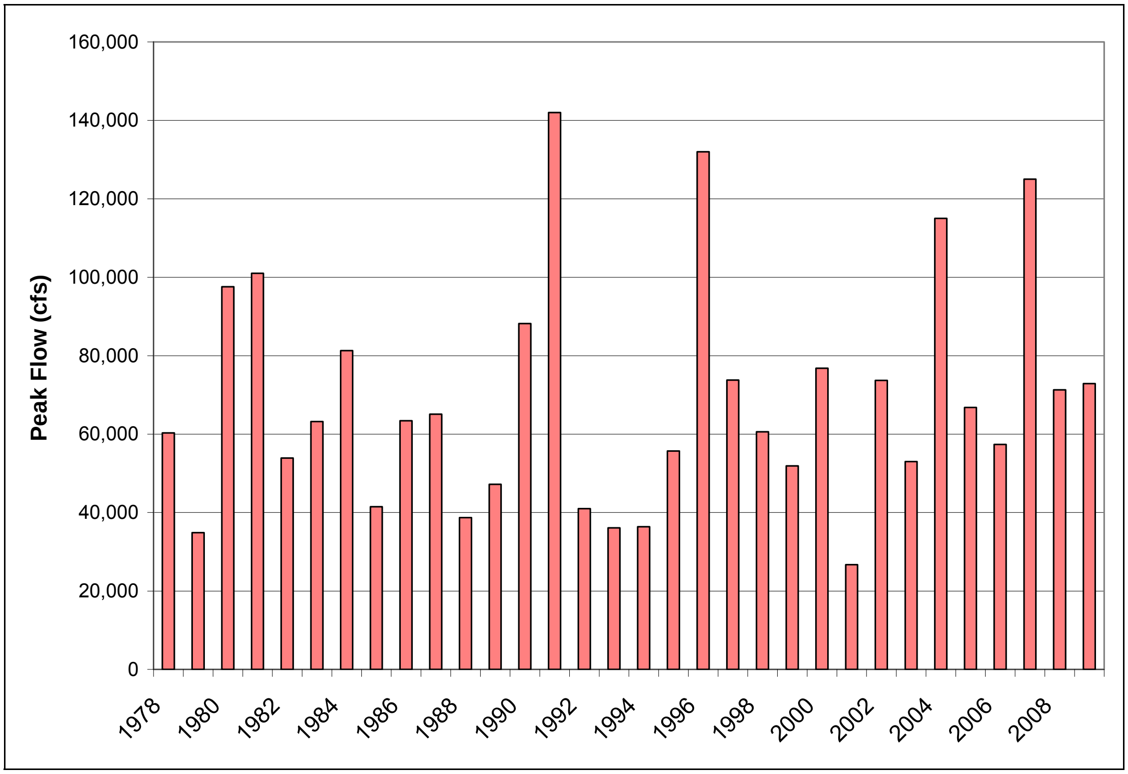


Figure 42. Annual peak flows (maximum daily) of the Skagit River at Mt Vernon, Washington; 1978 – 2009 (source: U.S. Geological Survey peak flow data for streamflow gage 12200500).

11.3.5 Trends in Freshwater Survival

Freshwater (egg-to-smolt) survival rates of wild Skagit steelhead ranged between 0.5 and 5.3% for the 1993 to 2007 outmigration years (Figure 43). Freshwater survival rates were lowest from 1993 to 1997. Poor freshwater survival rates in 1993 and 1994 likely reflect the impacts of a major flood event that occurred during the 1991 water year on juvenile steelhead survival, with discharge exceeding 140,000 cfs at the Mt Vernon gage during this event. Freshwater survival rates for wild steelhead increased from 2000 through 2005, exceeding 5% during the 2005 outmigration year. Freshwater survival rates dropped below 2% in 2006 and 2007.

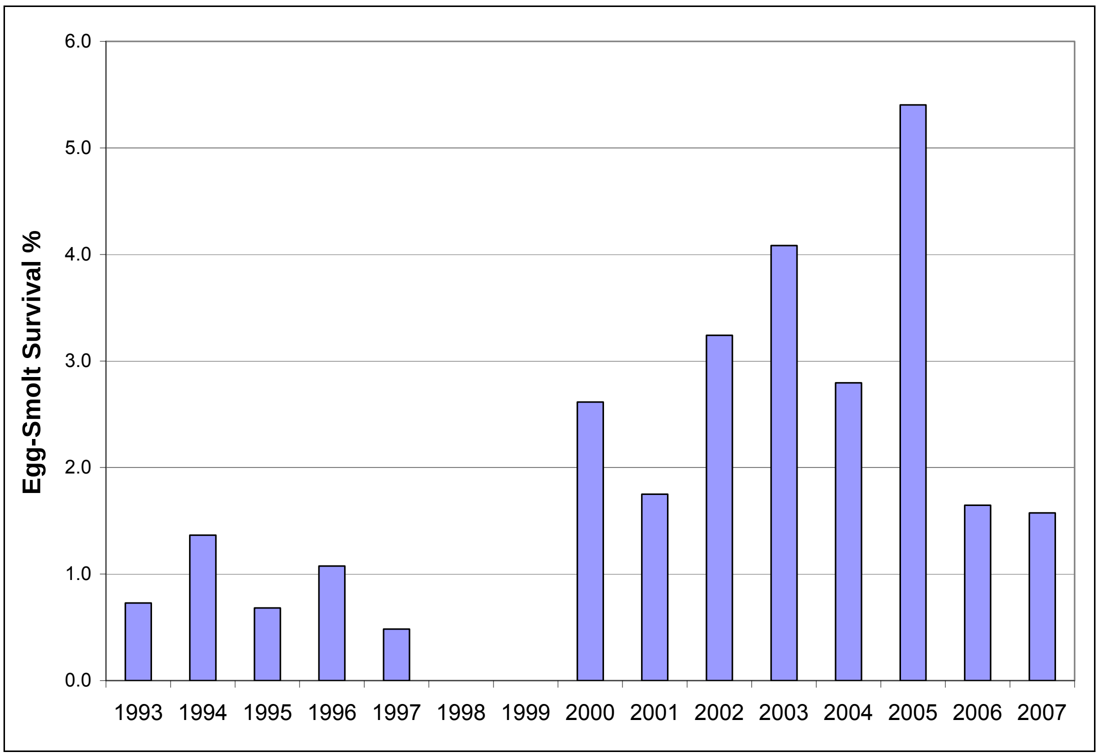


Figure 43. Annual egg-to-smolt survival rates calculated from escapement and smolt trap data for wild steelhead in the Skagit River; outmigration years 1993-2007. Freshwater survival rates could not be calculated for 1998 and 1999 due to the lack of escapement data for the 1996 and 1997 brood years.

As a result of relatively low freshwater productivity rates for wild steelhead in the Skagit during most of this period (< 5% egg-to-smolt survival), and due in part to elevated releases of hatchery steelhead that occurred during this same period, the number of hatchery smolts outpacing wild steelhead smolts in all but one year (Figure 44). While wild smolts varied considerably from 1993 through 2007 due to shifts in spawner abundance and freshwater survival, hatchery smolt release numbers remained relatively constant during this period.

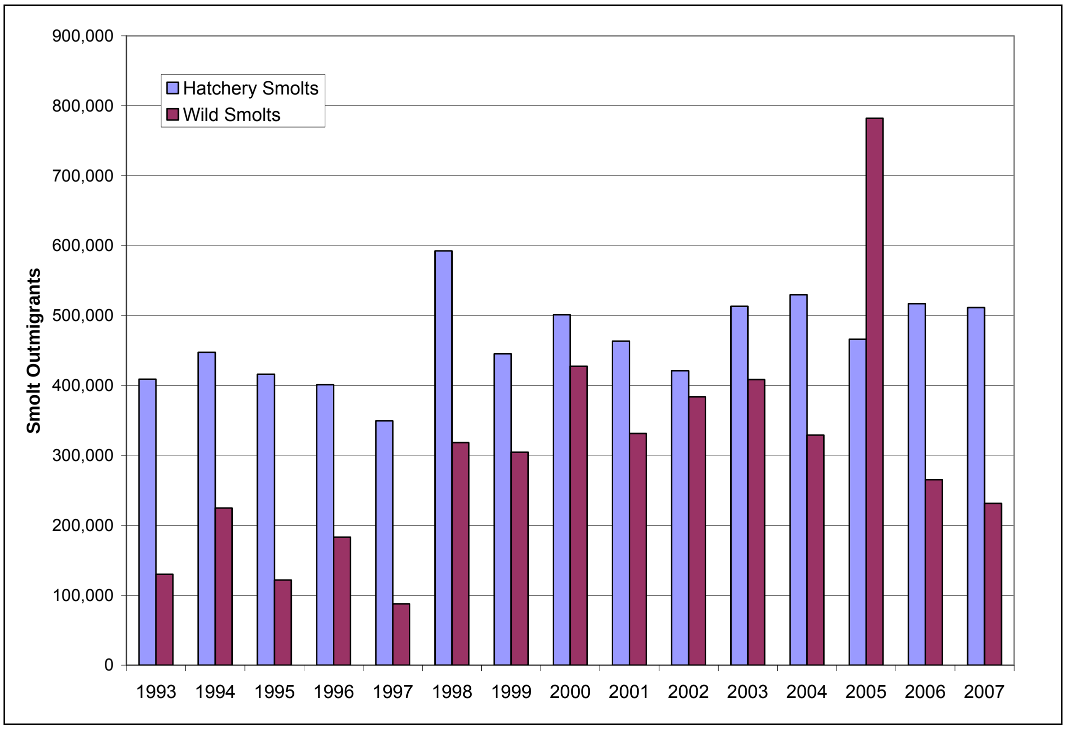


Figure 44. Estimated outmigration rates of wild steelhead smolts compared to releases of hatchery smolts in the Skagit River, 1993 – 2007.

11.3.6 Trends in Ocean Survival

The majority of wild and hatchery steelhead from the Skagit River spend either two or three years in the Pacific Ocean, with smaller proportion residing in the ocean for four years or more years (< 5% of returning adults). Due to their lengthy marine residency, shifts in ocean conditions and productivity can have major impacts on both wild and hatchery steelhead returns. The effects of ocean productivity cycles can have similar effects on wild and hatchery fish from the same basin, provided that they migrate to the similar areas in the north Pacific during their marine portion of the life cycle.

The effects of long-term cycles in ocean conditions on Skagit steelhead can be readily observed by examining trends in SAR rates for hatchery steelhead returns from 1978 through 2007 (Figure 45). SAR rates ranged from approximately 1.5 to 3.5 percent in the late 1970s and early 1980s, and then increased to a peak value of about 6% in 1985. SAR rates then declined rapidly from 1986 through 1994, when the SAR value dropped to 0.3%. SAR rates have remained very low for Skagit hatchery steelhead from the mid 1990s through the late 2000s, ranging from 0.2 to 0.8 percent. The low SAR rates observed in Skagit hatchery steelhead following the mid 1990s reflect a period of poor ocean survival conditions that has now occurred for almost 20 years.

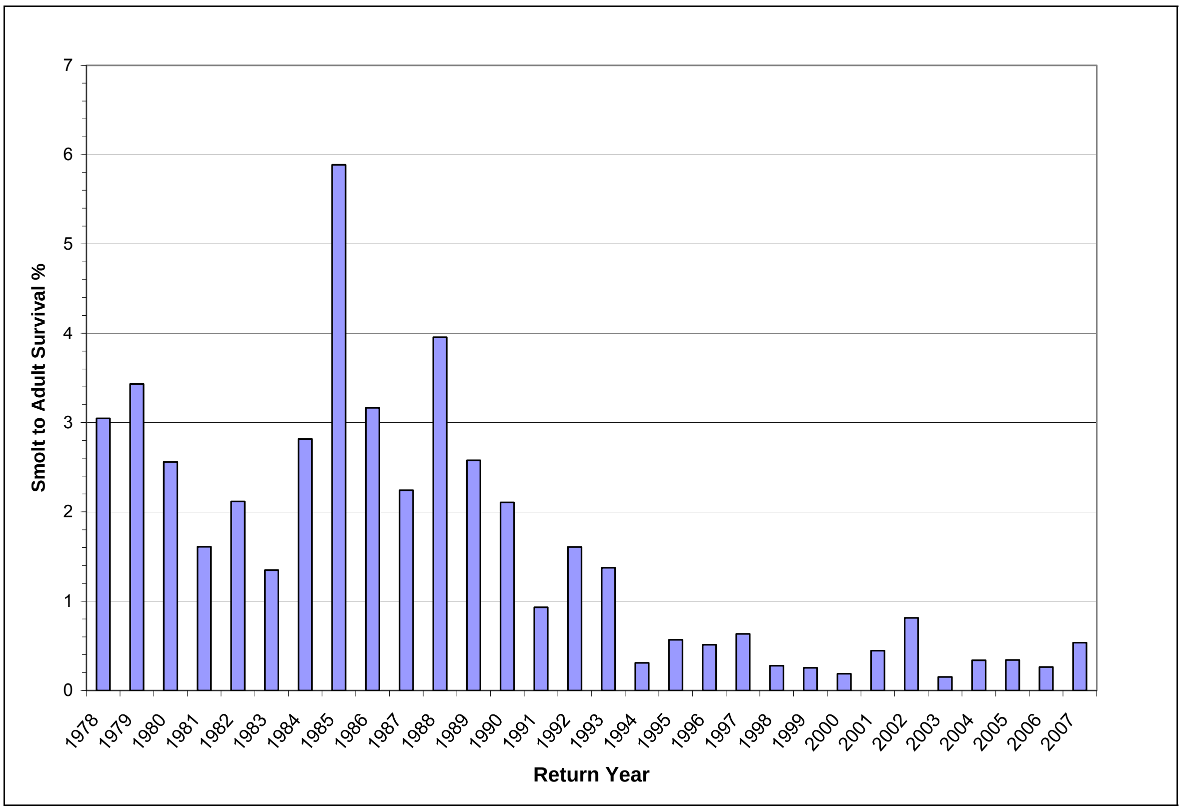


Figure 45. Smolt to adult survival (SAR) rates for hatchery steelhead in the Skagit River basin, 1978 – 2007 return years.

The SAR rates for wild Skagit steelhead were found to be substantially greater than those for hatchery fish from the Skagit over the same period (Figure 46). This finding suggests that marine survival rates are many times higher for wild steelhead than for hatchery steelhead in the Skagit. For the spawner return years 1997 through 2007 (the period of time for which smolt data is available from the Skagit smolt trap), marine survival rates for wild steelhead ranged from 0.8 to 6.6%, while marine survival rates for hatchery steelhead ranged from 0.2 to 0.8%.

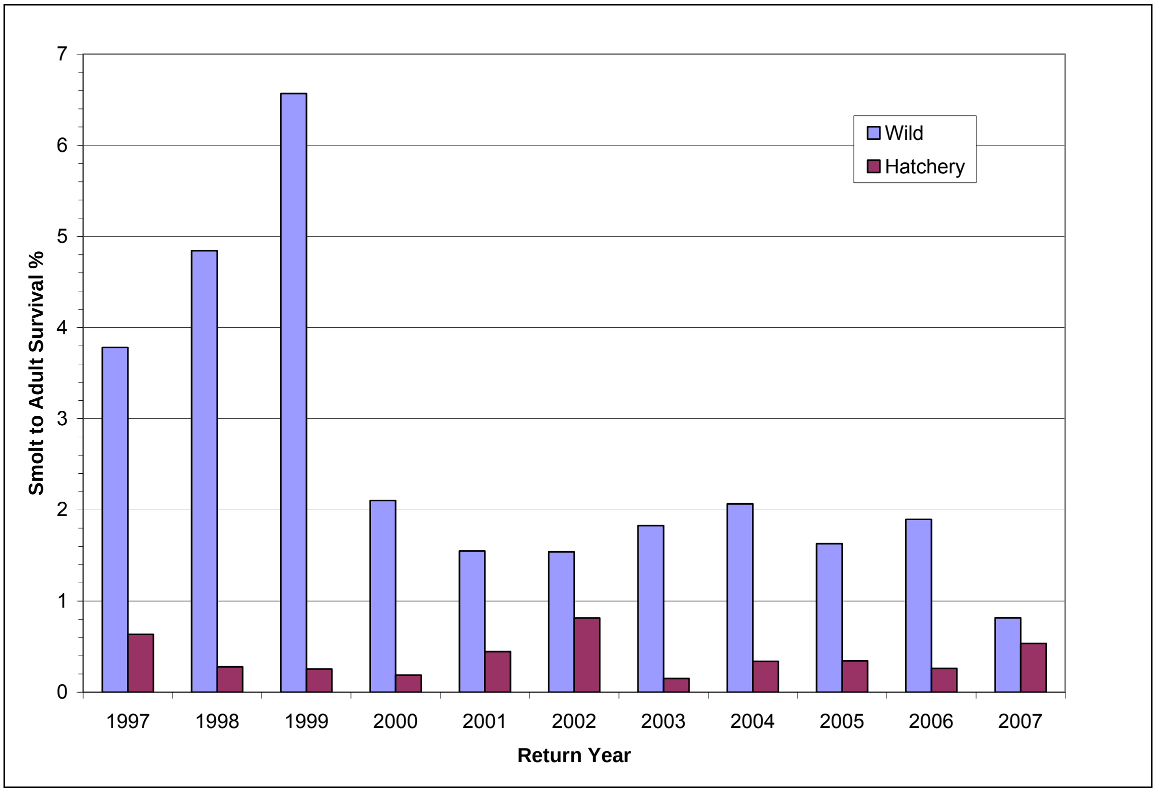


Figure 46. Smolt-to-adult (SAR) survival rates for wild and hatchery steelhead in the Skagit River basin, 1997 – 2007 return years.

11.3.7 Linear Regression – Effects of Hatchery Smolt Releases on Natural-Origin Steelhead Returns

We examined the potential relationship between trends in the number of hatchery smolts released into the Skagit River and wild steelhead return numbers. We assumed that the impacts of hatchery smolts on wild steelhead returns would accumulate over time. Any measurable effects of hatchery releases on steelhead returns would take at least one generation to detect assuming that hatchery fish were mainly impacting the abundance and freshwater productivity of wild juvenile steelhead. If this was the case, the ecological impacts of hatchery releases (e.g., competition for habitat and food; increased predation) on wild steelhead would not be detectable for three to seven years until the adults produced by these juveniles returned to the river. We assumed a mean generation time of five years for this analysis. Impacts to wild fish caused by spawning with hatchery spawners would not likely be detected for at least two generations, and impacts caused by genetic introgression (i.e., backcrossing of wild-hatchery hybrids with wild fish) would not likely be detectable for at least three or four generations.

We found that hatchery smolt releases had a strongly negative effect on wild steelhead returns for the past 30 years (Figure 47), and that this relationship was highly significant ($p < 0.01$). Hatchery smolt releases (15-year cumulative total) explained 35% of the variability in steelhead returns to the Skagit (R-Square = 0.353). This finding suggests that the hatchery release program in the Skagit has had a long-term, multi-generational impact on wild steelhead.

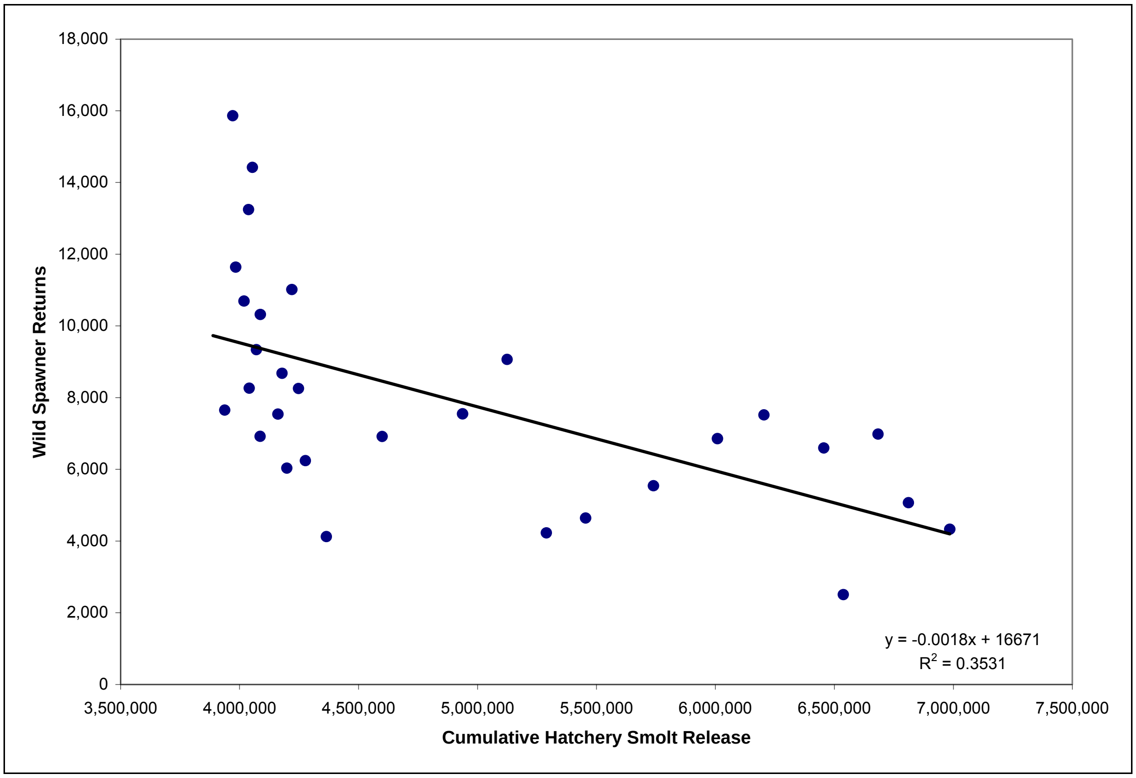


Figure 47. Relationship between cumulative hatchery smolt releases (15-year period) and wild steelhead returns for the Skagit River; 1978 – 2010. The trend line was plotted using a least-squared linear regression ($p < 0.01$; RSQ = 0.35).

11.3.8 Linear Regression – Influence of PDO on Skagit Natural-Origin Steelhead Returns

We conducted a linear regression analysis to assess the relative influence of a number of key environmental factors on wild steelhead returns in the Skagit. The objective of this analysis was to identify known sources of variability in wild steelhead returns so that we could better isolate the impact of hatchery releases on these returns. The examination of trends in marine survival (SAR) rates for wild steelhead strongly suggest that Skagit returns were being influenced by shifting ocean productivity regimes during their marine life history. To test this hypothesis, we examined the effect of the Pacific Decadal Oscillation (PDO), an index of surface sea temperatures in the North Pacific developed by the University of Washington (Hare and Mantua 1998), on annual return numbers of wild Skagit steelhead. We lagged the average annual PDO values by zero, one, two, and three years for the linear regression analysis to evaluate the influence of PDO conditions on different year classes of wild steelhead in the Skagit.

The results of the regression analysis found that changes in ocean conditions, as measured by the PDO index, have a significant effect on wild steelhead returns for the Skagit River (Figure 48). Steelhead returns were found to be most correlated with surface sea temperatures occurring two years prior to their return (i.e., PDO lagged by two years), meaning that ocean conditions were likely having the greatest impact on Skagit steelhead during their first year of ocean residency (note: the majority of Skagit steelhead reside in the ocean for two years). The PDO index was found to have a strongly positive effect on Skagit steelhead return numbers that was highly significant ($p < 0.001$).

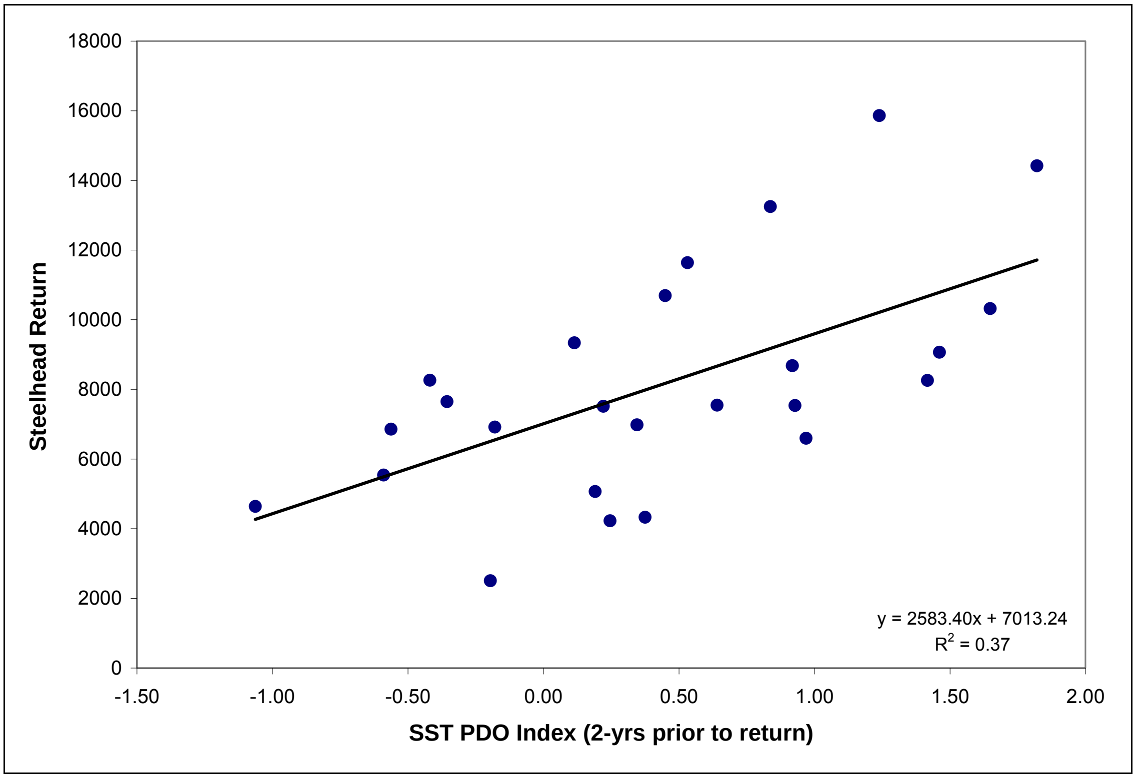


Figure 48. Relationship between PDO Index of surface sea temperatures (SSTs) and native steelhead returns in the Skagit River, 1978 – 2010. We used the mean yearly value of the PDO, which was then lagged by two years. The trend line was plotted using a least-squared linear regression ($p < 0.001$; RSQ = 0.37).

To corroborate the finding that ocean conditions have a strong effect on steelhead returns in the Skagit, we compared wild steelhead return rates in the Skagit with the best long-term steelhead data set in the British Columbia, the Keogh River. The Keogh is located on the northeast side of Vancouver Island, and is approximately 400 km northwest of the Skagit River. Steelhead return data for the Keogh River were obtained from McCubbing and Ward (2008). Steelhead returns in the Keogh River were found to be highly correlated with steelhead returns in the Skagit River (Figure 49); (R-Square = 0.69; $p < 0.001$). This correlation suggest that native steelhead in both river basins are tracking a common environmental factor, given the distance between these systems that factor is most likely shifts in ocean conditions.

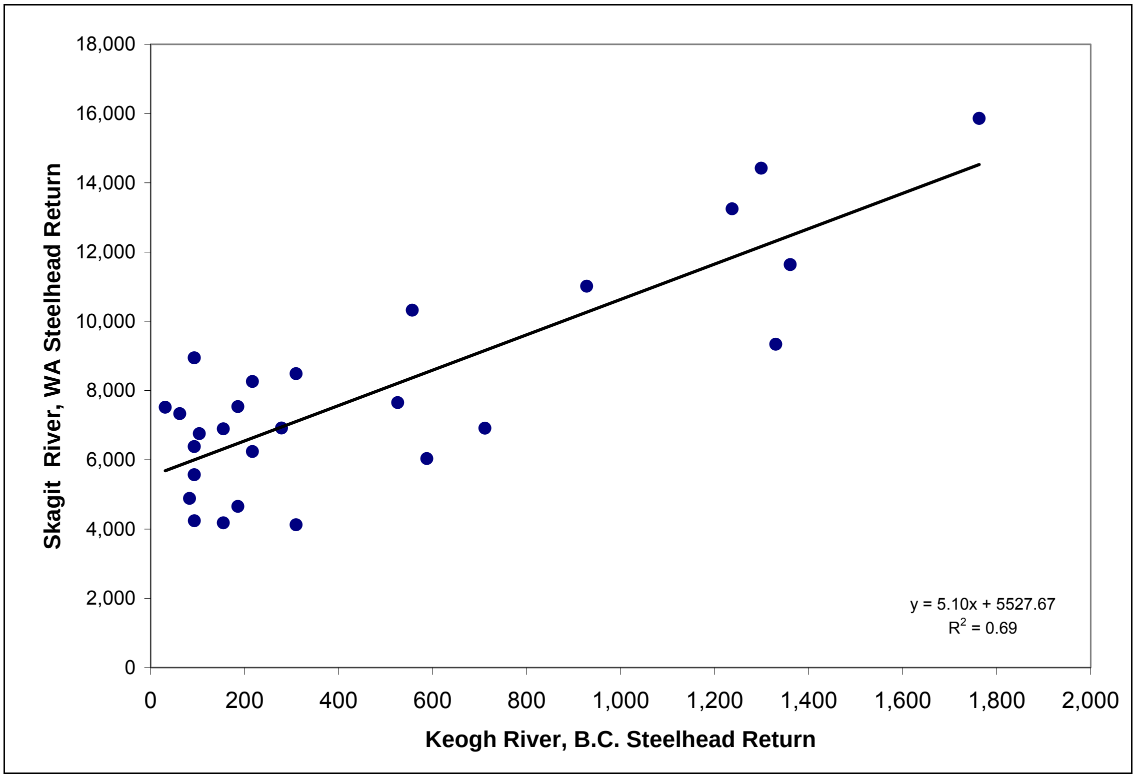


Figure 49. Linear regression relationship between steelhead returns for the Skagit River, Washington and Keogh River, British Columbia; 1978 – 2008 ($p < 0.001$; RSQ = 0.69). This relationship suggests a strong mutual dependency of these two steelhead populations on ocean conditions and regional climatic and hydrological factors.

11.3.9 Multiple Regression Model for Skagit Natural-Origin Steelhead Returns

The negative correlation observed between wild steelhead returns and hatchery steelhead release numbers provides indirect evidence that hatchery steelhead are having a negative impact on wild steelhead. However, it is possible that this correlation could actually reflect the effects of another "hidden" variable that is inter-correlated to hatchery release numbers over time. For example, the hatchery release data for the Skagit suggests that hatchery steelhead hatchery production increased over the past 15 years to compensate for poor hatchery adult returns. Assuming that the poor returns were related to shifts in ocean conditions as measured by the PDO, then hatchery releases numbers and annual PDO values could conceivably be highly correlated. Thus, changes in the PDO, not hatchery release numbers, could be the causal factor impacting wild steelhead returns.

The subset of variables that best explained wild steelhead returns to the Skagit River were the PDO index, peak flows measured at the Mt Vernon gage, and hatchery steelhead smolt releases (Table 39). This multiple regression was highly significant ($p < 0.001$), and explained 69% of the variability in wild steelhead returns (adjusted R-Square = 0.69). The PDO index had a positive effect while peak flows and hatchery smolt releases a negative effect on wild steelhead returns to the Skagit (Table 39). The effect of all three independent variables on steelhead returns was highly significant ($p < 0.001$ for PDO index and hatchery smolt releases; $p < 0.01$ for peak flows).

Review of the standard regression coefficients for the independent variables show that the PDO index had the largest effect on wild steelhead returns, followed by hatchery smolt releases and peak flows (Table 39). The tolerance values for the independent variables in this multiple regression model were found to substantially exceed the 0.10 threshold below which multicollinearity is considered problematic (Hair et al. 1995). Thus, the three independent variables can be considered to be statistically independent. This means that effects of hatchery smolt releases can be considered to be separate and distinct from the effects of the PDO index (ocean conditions) and peak flows (freshwater conditions) on wild steelhead returns in the Skagit.

Table 39. Multiple regression analysis of Skagit River native steelhead returns as determined by the PDO Index (lagged 2 years), annual peak Skagit River flows (lagged 3 and 4 years), and hatchery smolt release numbers (15-year moving average); 1978-2010. PDO Index values were multiplied by 100 and smolt release numbers divided by 1,000 for standardization. The R-Square for the regression is 0.69 ($p < 0.001$). Interactions among the independent variables were not significant.

Effect	Regression Coefficient	Standard Error	Standard Coefficient	Tolerance	t-Value	p-Value
Constant	16,660	1,955	0		8.52	0.000
PDO Index	2.707	0.54	0.603	0.819	5.017	0.000
Peak Flow	-0.044	0.013	-0.394	0.892	-3.424	0.002
Smolts Released	-1.230	0.344	-0.409	0.902	-3.576	0.001

11.3.10 Regional Analysis of Hatchery Smolt Effects on Steelhead Productivity

The mean annual escapement values for wild steelhead among the Puget Sound river basins over the past 5 years (2006-2011) ranged from less than 100 spawners in Tolt and Tahuya, to over 4,200 spawners in the Skagit (Figure 50). Populations of steelhead have substantially declined in the majority of these systems since the mid-1980s (Hard et al. 2007; Ford 2011).

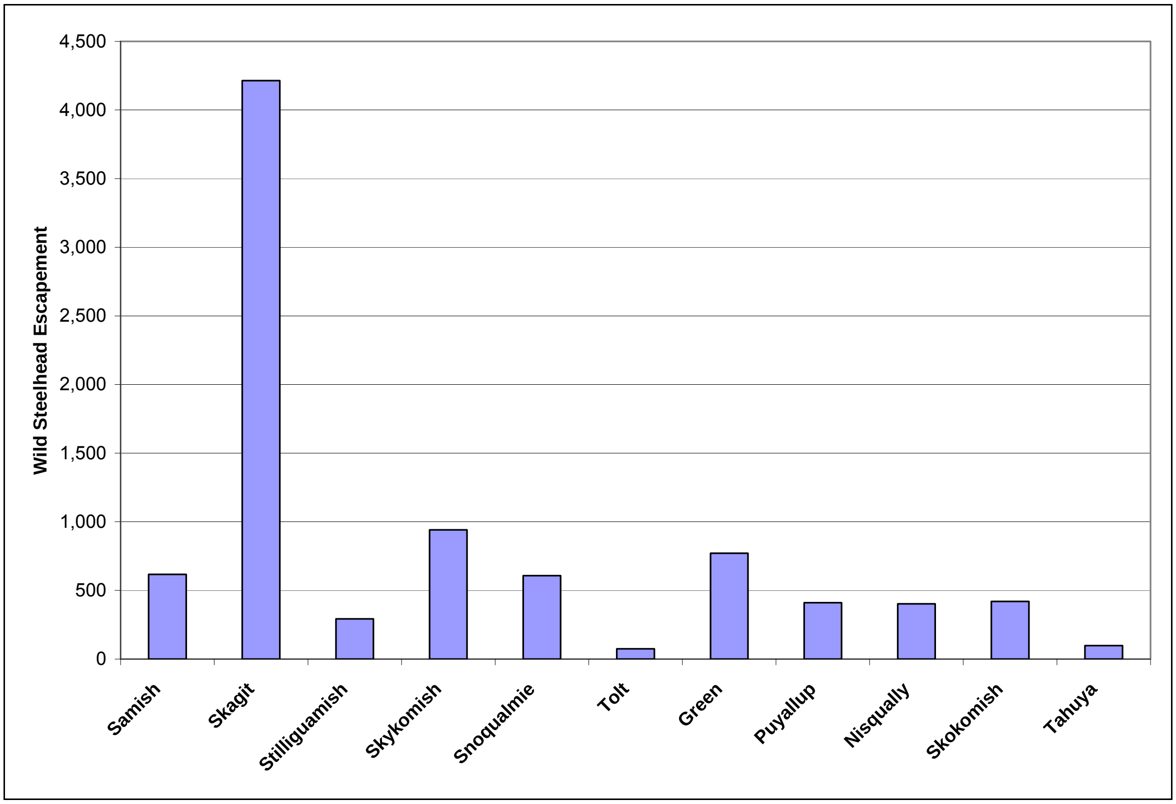


Figure 50. Mean annual escapement numbers for wild steelhead in Puget Sound watersheds over past five years (2006-2011).

Wild steelhead spawner densities were quite variable for the Puget Sound watersheds we analyzed, ranging from 0.2 spawners per sq-km in the Puyallup River to 2.7 spawners per sq-km in the Samish River (Figure 51). All of the rivers except the Samish had spawner densities less than 1.0 spawners per sq-km, which reflects the historically low population levels in steelhead that have been observed over the past decade. The Skagit had the highest spawner density value among the large river systems evaluated in the Puget Sound. The Stillaguamish, Puyallup, and Nisqually rivers had the lowest spawner densities.

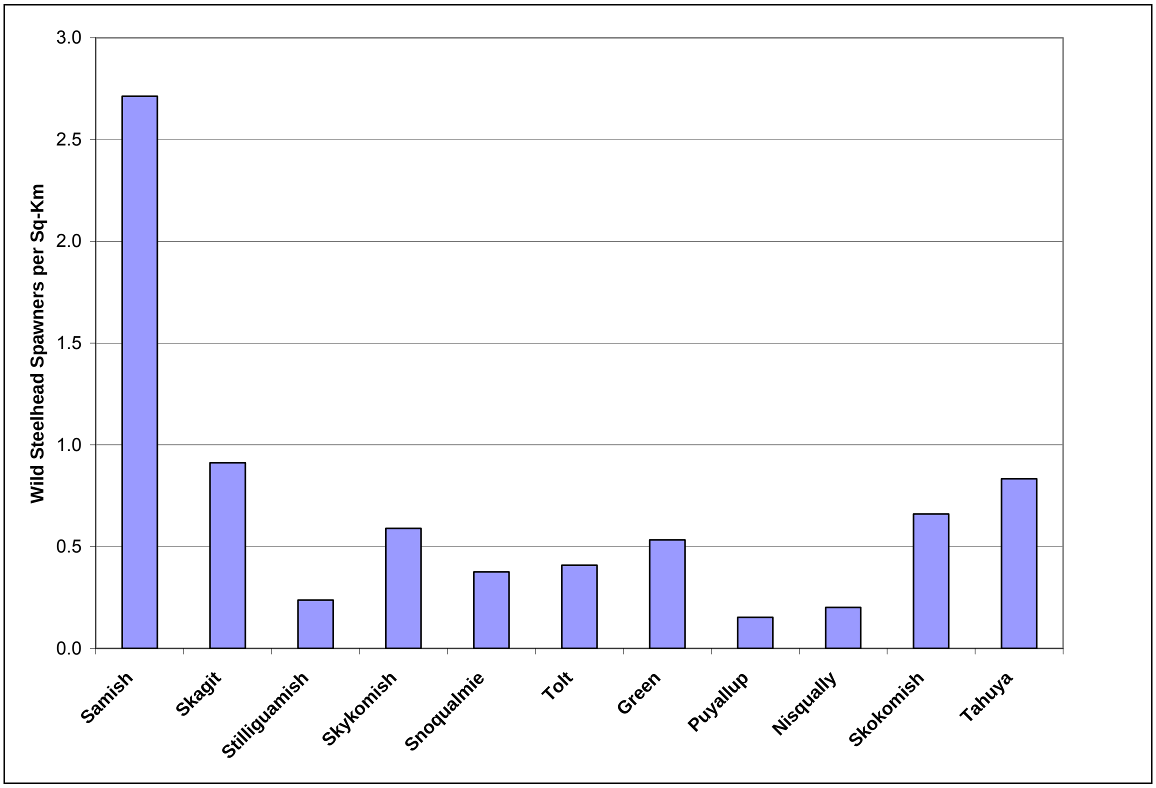


Figure 51. Mean spawner densities (escapement per watershed area) for the past five years (2006-2011) among Puget Sound watershed (source: WDFW SASSI database 2012).

More hatchery steelhead smolts were released into Skagit River than any other river we evaluated in the Puget Sound (Figure 52). For the past 30 year, the average release of hatchery smolts into the Skagit has been 364,000 fish per year. Most of the hatchery fish released into the Skagit were winter run fish, though some summer run fish have been introduced into this system. In addition to the Skagit, hatchery releases were relatively high in the Stillaguamish, Skykomish, Snoqualmie, Green, and Puyallup rivers. The proportion of summer-run steelhead released into these systems was highest in the Skykomish River.

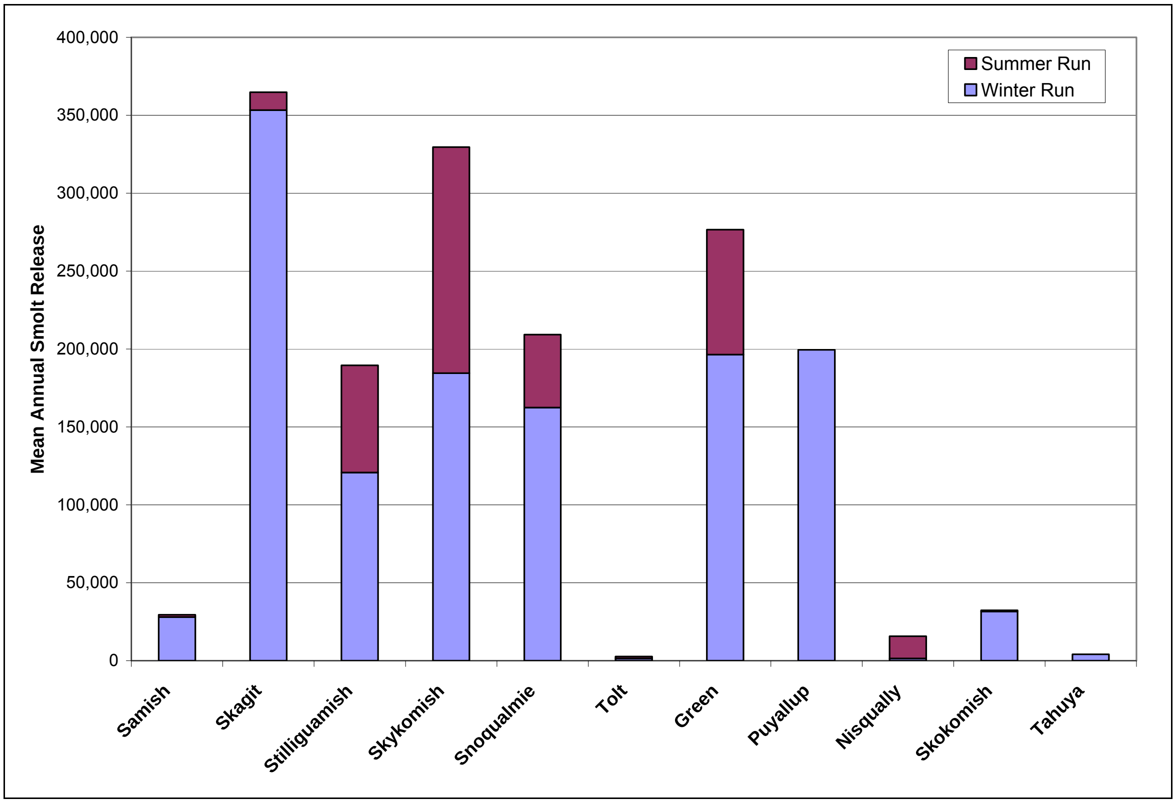


Figure 52. Mean annual release numbers of winter and summer hatchery smolts in Puget Sound watersheds over last 30 years (1971-2010).

The Skykomish River had the highest number of hatchery smolts released per square-kilometer of watershed area, with an average annual release of 207 smolts per sq-km (Figure 53). The Stillaguamish and Green river watersheds also had high densities of smolts released, with 154 and 191 smolts per sq-km released per year, respectively. The Skagit River had a moderate level of smolts released per watershed area, with an average of 79 smolts released per sq-km per year. The Tolt, Nisqually, and Tahuya rivers had the lowest releases of smolts per watershed area over the past 30 years, with less than 40 hatchery smolts released per sq-km in these watersheds per year (Figure 53).

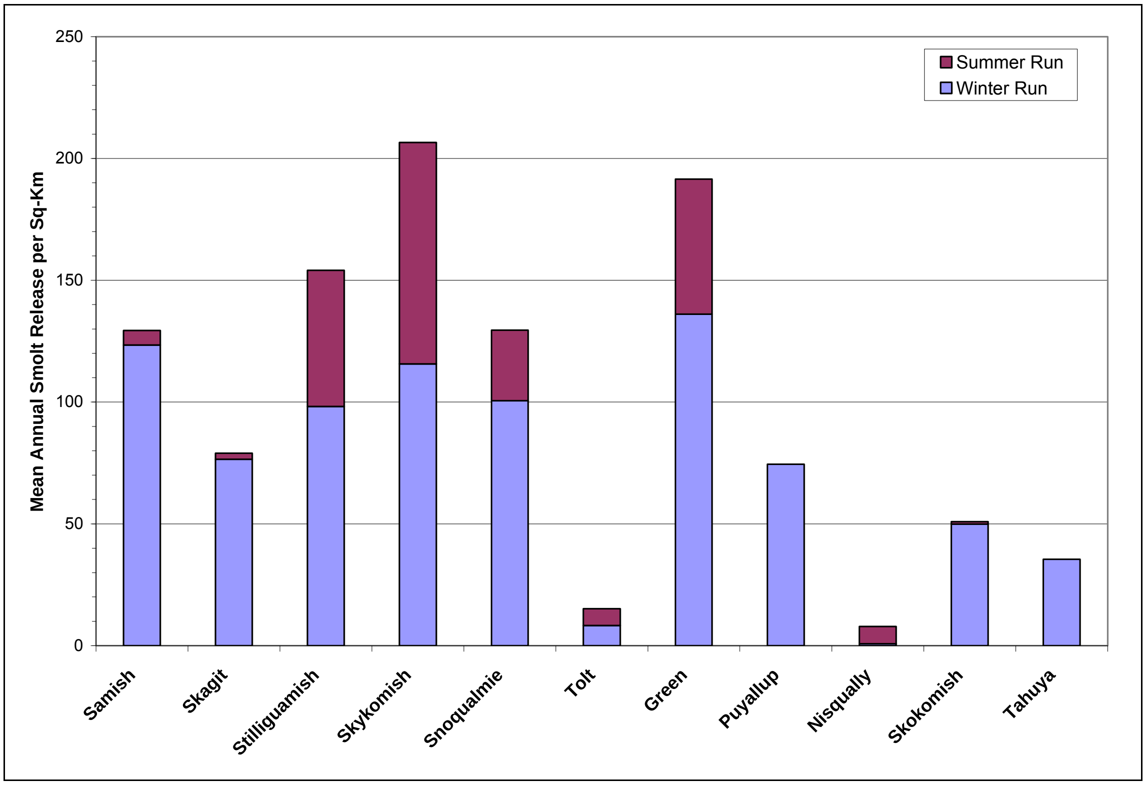


Figure 53. Mean annual hatchery steelhead smolts released per square-kilometer for Puget Sound watersheds over last 30 years (1971-2010).

We found that population growth rates of wild steelhead significantly declined as the number of hatchery smolts per spawner increased (Figure 54). The highest population growth rates for wild steelhead were observed in watersheds having the lowest number of hatchery smolts released per wild spawner, including the Samish, Tahuya, Tolt, Skagit, and Skokomish rivers basins. With the exception of the Nisqually River, the lowest population growth rates for wild steelhead were found in those watersheds having the highest number of hatchery smolts released per wild spawner (i.e., Puyallup and Stillaguamish rivers). Wild steelhead growth rates were intermediate in those watersheds that had moderate smolt released numbers per spawner, namely the Snoqualmie, Skykomish, and Green watersheds. This regression relationship was statistically significant ($p < 0.05$), and explained 40 percent of the variability in the population growth rates observed among Puget Sound watersheds between 1985 and 2011. Population growth rates (lambda) were negative (i.e., declining escapement) in all watersheds except the Samish during this period.

The Nisqually River watershed was an outlier in this relationship, and was the only watershed with low hatchery smolt release numbers that also had a low population growth rate (Figure 54). There are several possible explanations for the low population growth rate observed in the Nisqually River, including low juvenile survival rates caused by the poor habitat and water quality conditions present in the southern Puget Sound. Assuming that the Nisqually River is an outlier based upon local environmental conditions, we conducted the regression analysis between hatchery smolt releases and wild steelhead population growth rates without the Nisqually River data. The resulting statistical relationship was highly significant ($p < 0.001$), with the number of hatchery smolts released per wild spawner explaining 82 percent of the variability in wild steelhead growth rates among the Puget Sound watersheds included in this analysis (Figure 54).

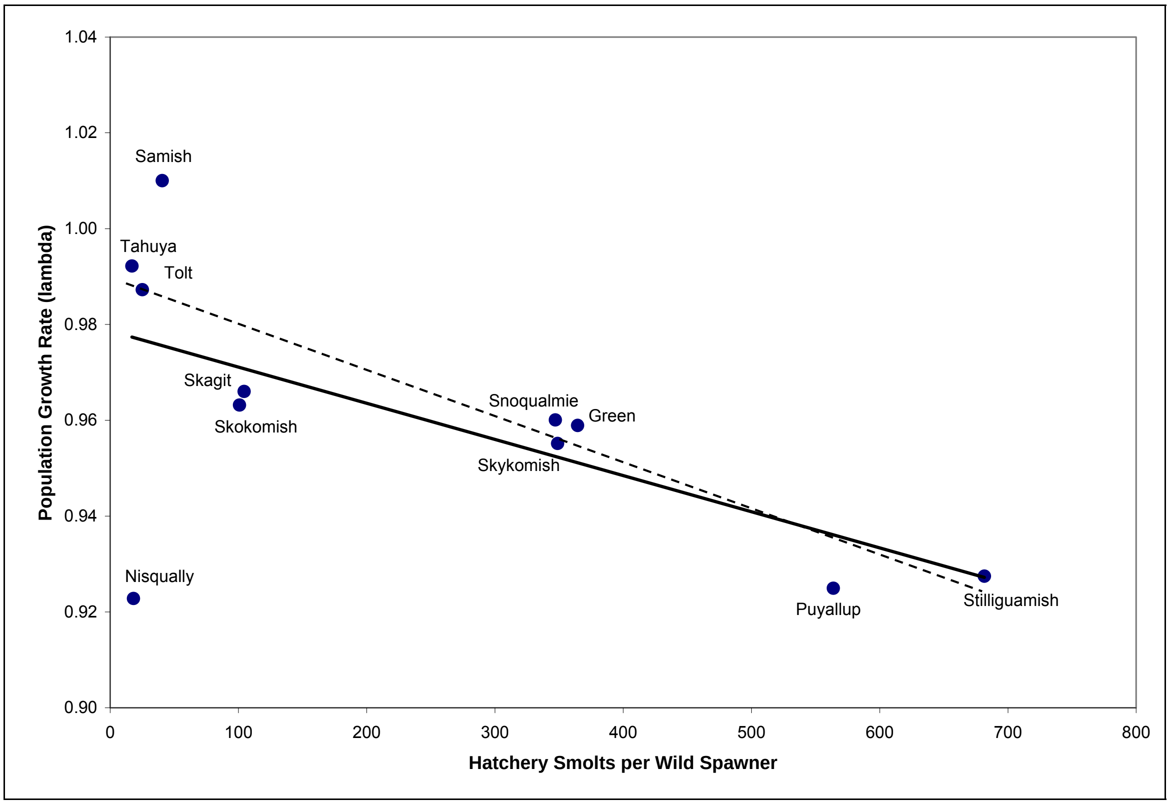


Figure 54. Linear regression relationship between hatchery smolt releases and population growth rates for native steelhead among Puget Sound watersheds (solid line; $p < 0.05$; RSQ = 0.40). Hatchery smolt releases are 15-year average for combined winter and summer steelhead stocks. Population growth rates (lambda) for each watershed were calculated for 1985-2011 using exponential growth fit model. Dashed line shows relationship after removal of Nisqually outlier ($p < 0.001$; RSQ = 0.81).

11.4 Discussion/Conclusions

11.4.1 What Factors Affect Natural-Origin Skagit Steelhead Returns?

We found that there is a combined influence of three independent variables - Skagit hatchery smolt release numbers, surface sea temperatures in the North Pacific, and peak flows in the Skagit – on native Skagit steelhead adult returns. This analysis also allowed us to identify the relative effect of hatchery smolt release numbers on adult native returns while controlling for the influence of the other two independent variables. This analysis found that all three independent variables (smolt release numbers, surface sea temperatures, and Skagit peak flows) contributed significantly to native steelhead returns. Moreover, inter-correlations among the three independent variables were found to be very weak, meaning that the statistical effects of each variable were not being influenced by the other variables. Consequently, hatchery smolt releases were found to have a highly significant and negative effect on native steelhead returns in the Skagit that was independent of long-term trends in marine and freshwater conditions.

The inverse relationship between hatchery smolt releases and wild steelhead returns that is observed when examining the time-series plots of these two variables over the past 30 years is potentially the result of negative biological interactions occurring between hatchery and wild steelhead, including increased juvenile competition, increased susceptibility to predation by predators such as bull trout, lost spawning effort when wild fish spawn with hatchery fish, and reduced fitness when wild fish hybridize with hatchery fish that have substantially reduced marine survival rates. Hatchery smolts can have a number of negative impacts that increase the genetic and ecological risks to native steelhead populations (Kostow 2009), and which can reduce the abundance and productivity of these native populations over time (Chilcote 2003; Chicote et al. 2011; Kostow and Zhou 2006).

While hatchery steelhead in the Skagit have had an numerical advantage in the freshwater environment over the past 15 years due to the release of large numbers of hatchery fish, wild steelhead have the distinct advantage of being able to produce a far greater number of spawners for the same number of smolts (higher productivity). Wild steelhead smolts have produced between 2 and 20 times more adults than hatchery smolts since 1997 (see Figure 46). The very low SAR rates observed in hatchery steelhead in other Puget Sound rivers during this same period (WDFW 2008) suggests that Chambers Creek hatchery stocks are not well adapted to the poor ocean conditions that have occurred in recent years. This is similar to the finding on the survival rates of natural spawning hatchery steelhead in the Clackamas River, Oregon, which were found to have adult return rates that were ten times lower than native wild steelhead (Kostow et al. 2003).

Both wild steelhead and hatchery steelhead smolts in the Skagit have an average length of 180 mm (WDFW smolt trap data), suggesting that the difference in marine survival cannot be explained by smolt size alone. There are several possible explanations for the higher survival rates of wild over hatchery steelhead in the marine environment: 1) wild have higher survival rates while migrating through the Puget Sound, and are less susceptible to predation; 2) wild steelhead migrate to more productive areas of the north Pacific than hatchery fish; and 3) wild steelhead are better adapted to local conditions in the marine environment, and therefore have a greater ecological fitness to marine conditions than hatchery fish. While the specific reason for this difference in marine survival remains unclear, our analysis suggest that wild fish have a selective advantage over hatchery fish in terms of their population productivity as a result of substantially greater marine survival rates.

The regional analysis on the effects of hatchery smolt releases on native steelhead productivity among Puget Sound watersheds suggest that hatchery releases have had a long-term negative impact on steelhead population growth rates. Although differences in habitat quality are likely a major factor explaining the variability in population growth rates (i.e., productivity) among Puget Sound watersheds, the results of this analysis suggest that hatchery practices also have a significant influence on productivity patterns of wild steelhead in this region. This further corroborates our finding that hatchery steelhead releases have had a long-term impact on native steelhead returns to the Skagit River basin.

11.4.2 Potential Mechanisms That Can Affect Natural-Origin Skagit Steelhead Returns

Hatchery releases - The results of our multiple regression model provides evidence that there is a cause-and-effect relationship between increasing hatchery releases and declining steelhead returns in the Skagit River. Long-term releases of hatchery smolts were found to have a negative and highly significant effect on native steelhead returns to the Skagit which was independent of the effects shifting ocean and freshwater conditions. The likelihood of a cause-and-effect relationship between hatchery releases and wild steelhead populations is further supported by the findings of the genetic analysis conducted for this report, which suggests that juvenile steelhead with various levels of hatchery-origin ancestry are present throughout the rearing areas for wild steelhead in the Skagit basin. The widespread presence of juveniles with hatchery ancestry suggests that ecological impacts on wild juvenile fish, including that of competition for habitat and food, are likely. The genetic analysis presented in other chapters of this report also suggest that hatchery fish, or their offspring, are spawning with wild fish in the Skagit basin. This would result in lowered population productivity rates for wild steelhead, given that hatchery fish have substantially lower marine survival rates than wild fish.

Studies designed to determine wild and hatchery Pacific salmon productivity date back to 1931 when several methods of hatchery propagation were found to be less productive than natural propagation for wild sockeye salmon at Cultus Lake of the lower Fraser River (Foerster 1931). There have been numerous studies indicating that hatchery steelhead do not perform well in the wild and can have subsequent negative effects on wild steelhead dating from the 1970s to the present (Reisenbichler and McIntyre 1977; Chilcote et al. 1986; Araki 2007-a; Araki 2007-b; Araki 2008; Araki et al. 2009; Seamons et al. 2012; Byrne and Copeland 2012; and Christie et al. 2012) and this can occur in a very short time (Christie et al. 2011). There have also been broader overviews of the range of consequences and mechanisms affecting wild populations as a result of hatchery fish (Flagg et al. 2000; Kostow 2008; Jonsson and Jonsson 2006; Grant 2012; Morrison 2012). Specific concerns about Washington's hatchery steelhead program's effects on the wild steelhead populations date to 1972 (Royal 1972). Some of the more specific mechanisms through which hatchery fish have negative consequences on wild fish populations as indicated in differing studies and reports are provided in Table 40 (McMillan 2012).

Table 40. Potential mechanisms of hatchery impacts on steelhead populations described in literature.

Potential mechanism	References
Spawning interactions, genetic hybridization	Adults (Reisenbichler and McIntyre 1977; Reisenbichler and Rubin; Seamons et al. 2012) Precocious male parr (McMichael et al. 1999; Tipping et al. 2003; McMillan et al. 2007; McMillan et al. 2011; Christie et al. 2011-a)
Unintended Straying to Natural Spawning Grounds; and Lack of Spawn Time Separation between Wild and Hatchery Steelhead Selected to Be Different	Straying (Shapovalov and Taft 1954; Lirette and Hooton 1988; Schroeder et al. 2001; Jonsson et al. 2003; Keefer and Caudill 2012; Seamons et al. 2007; Seamons et al. 2012) Lack of Spawn Time Separation between Wild and Hatchery Fish Selected to be Different (Mackey et al. 2001; Seamons et al. 2012)
Reduced Fitness and/or Reproductive Success	(Reisenbichler and McIntyre, 1977; Close 1999; Kostow and Zhou 2006; Araki et al. 2007-a; Araki et al. 2007-b; Araki 2008; Araki et al. 2009; Chilcote et al. 1986; Chilcote et al. 2011; Christie et al. 2011; Bernston et al. 2011; McLean et al. 2003; Seamons et al. 2012; Byrne et al. 1992; Byrne and Copeland 2012; Christie et al. 2012)
Reduced or Altered Life Histories	(Jonsson and Jonsson 2006; Zaporozhets and Zaporozhets 2012; Miyakoshi et al. 2012)
Competition/Density Dependence	(Berejikian et al. 1996; McMichael et al. 1999; Kostow and Zhou 2008; Levin et al. 2001; Pearson et al. 2007; Ruggerone et al. 2012; Zhivotovsky et al. 2012) A sub-mechanism in this category is residualization of hatchery smolts (Royal 1972; Cannamela 1992; Viola and Schuck 1995; Tipping et al. 1995; McMichael et al. 1997; McMichael et al. 1999; Washington Trout 2004)
Indirect Predation	The relationship of hatchery releases and predator attraction (Thompson and Tufts 1967; Beamish et al. 1992; Nicholson 2003; Balfry et al. 2011; Collis et al. 1995; Einum and Fleming 2006; Handelmann et al. 1996; Steward and Bjornn 1990)
Overharvest in Mixed Stock Fisheries	(Flagg et al. 1995; Larkin 1977; Wright 1993)

Surface Sea Temperatures - The strong statistical relationship between Skagit wild steelhead returns and the PDO suggests that steelhead returns are in part influenced by environmental conditions in the North Pacific. This relationship suggests that returns in wild Skagit steelhead increase following periods of warm surface sea temperatures in the North Pacific, while returns decline following periods of cool surface sea temperatures. The PDO index increases as surface sea temperatures in North Pacific become warmer (Hare and Mantua 2000).

Cooler water temperatures in the north Pacific have been associated with increased recruitment (hence increased ocean survival) of Chinook, sockeye, and coho salmon (Mantua et. al 1997). In contrast, the largest return of Skagit steelhead observed in the last 30 years occurred in the late 1980s following an extended period of warm sea temperatures in the north Pacific (as measured by strongly positive PDO index values). The major decline in Skagit steelhead returns observed during mid-2000s occurred following a period of cool surface sea temperatures (negative PDO index values) in the late 1990s and early 2000s (see Figure 41). This means that Skagit steelhead returns are positively correlated with warm surface sea temperatures, rather than the cool regimes that have been associated with strong returns of Chinook, sockeye, and coho salmon. This relationship supports the findings of research by Atcheson at the University of Washington (Atcheson 2010; Atcheson et al. 2012), who found that growth rates of steelhead significantly increase during periods of warm surface sea temperatures in the North Pacific. Our work showed a highly significant effect of ocean conditions two-years prior to spawning means that shifts in ocean temperature have their greatest influence during the first year of ocean residency for Skagit steelhead. This finding is supported by Atcheson's (2010) research on steelhead growth rates in the North Pacific. This study found that the correlation between the PDO and steelhead growth rates was greatest during the first year of marine residency.

This relationship suggests that steelhead populations in both the Keogh and Skagit rivers are responding to the same large-scale environmental variables, most probably a combination of ocean conditions in the North Pacific and regional hydrological conditions. The relationship implies that almost 70% of the variability in wild steelhead returns in these two rivers can be attributed to external large-scale environmental variables, rather than to local environmental variables within the two watersheds.

The long-term decline in SAR rates for hatchery steelhead is not unique to the Skagit, but has also been observed in other Puget Sound rivers including the Puyallup and Elwha (WDFW 2008). The average SAR index for winter-run (Chambers Creek origin) hatchery steelhead in the Puget Sound has declined from a peak of 7.0% in the mid 1980s to less then 0.4% through the 2000s. The SAR rates observed for Puget Sound steelhead hatchery stocks have remained the lowest among artificial steelhead production programs in the State of Washington since the mid 1990s (Scott and Gill 2008).

These marine survival rates are very low compared to average survival rates measured for wild steelhead in long term studies conducted in the Georgia Basin. With the exception of the Snow Creek monitoring study on the Hood Canal, there have been no long-term monitoring efforts for wild steelhead freshwater survival in the Puget Sound. The Snow Creek steelhead population does not correlate well with long term abundance trends for wild steelhead in the Skagit. However, there is a high degree of correlation between wild steelhead populations in the Skagit and Keogh River in the northern Georgia Basin (to be discussed later), with the Keogh River providing a high quality long-term dataset for wild steelhead in British Columbia (McCubbing and Ward 2008).

Peak flows - Peak flows have been found to significantly reduce the egg-to-smolt survival of Chinook salmon in the Skagit River (Kinsel et al. 2008), with survival declining when daily average flows measured at the Mt Vernon gaging station exceed approximately 50,000 cfs. Above 50,000 cfs, egg-to-smolt survival of Chinook salmon declines in direct proportion to peak annual flow. Declines in egg-to-smolt survival are greatest when peak flows exceed 80,000 cfs at Mt Vernon (Kinsel et al. 2008). Steelhead redds are not as susceptible peak winter flows as Chinook salmon, because steelhead spawn in the spring rather than in fall like Chinook (the majority of peak flow events occur from October through December in the Skagit based upon USGS flow data). However, steelhead juveniles are probably much more susceptible than juvenile Chinook to peak flows because the majority of steelhead juveniles rear in the Skagit for two to three years, while most Chinook juveniles have a relatively short freshwater rearing period that last from one to five months. A major flood event on the Skagit during the winter can impact three or more year classes of steelhead juveniles due to their extended freshwater rearing period.

11.4.3 Examples of Hatchery Mechanisms

How do hatchery fish reduce production of wild fish over time?

Figure 54 shows the negative relationship between hatchery smolt releases and wild steelhead returns over the past 32 years. Numerous factors have already been identified such as increased juvenile competition, increased susceptibility to predation by predators such as bull trout, lost spawning effort when wild fish spawn with hatchery fish, and reduced fitness when wild fish hybridize with hatchery fish that have substantially reduced marine survival rates. We also know that there is a cumulative effect of reduced fitness in steelhead hatchery operations as has been well documented (Araki 2007, 2008). The question then becomes how do hatchery fish potentially reduce production of wild fish over time?

The answer to this question is likely a combination of all the preceding factors. The average percentage of hatchery run-sizes to total run-sizes for Skagit basin hatchery winter-run steelhead between 1991 and 2011 was half of the average for the period between 1978 and 1990 with a resulting increase in the wild run-size proportion of total run-sizes (Figure 55, see McMillan 2012; Figure 20). Yet the resulting negative correlation for hatchery run-sizes to hatchery smolt plants was weaker than for wild run-sizes.

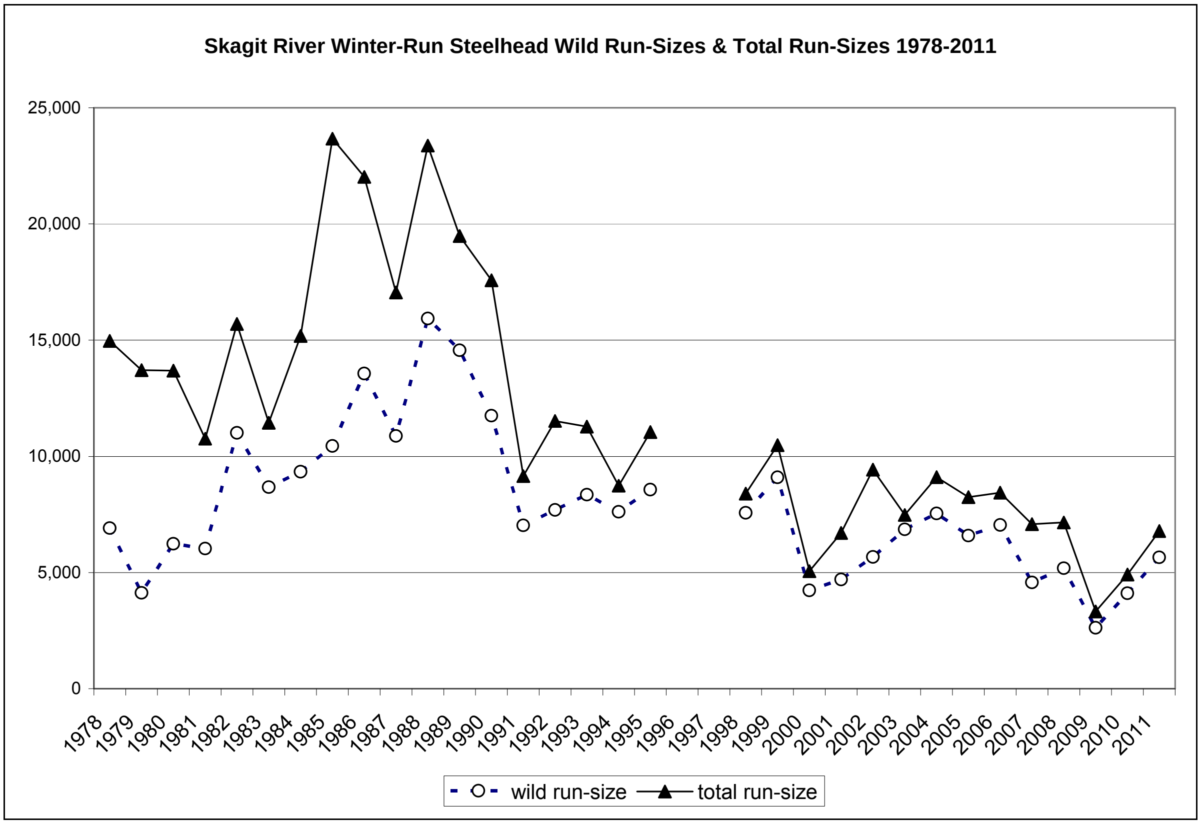


Figure 55. Skagit River winter-run steelhead. Native run sizes v. total run sizes, 1978 to 2011.

This is potentially explained by a similar, but longer term portrayal of the declining trend of hatchery steelhead returning to Chambers Creek hatchery between 1955 and 1997 culminating in cessation of the steelhead operations (Cooper and Johnson 1992; Eltrich 2007; McMillan 2012). This was apparently for lack of returns despite increased hatchery smolt plants from at least the early 1980s through 1990 (end of the graph examined). Return rates similarly declined since about the latter 1970s with a rapid drop in the early 1990s. Although unproductive ocean conditions have been used to explain the Chambers Creek hatchery declines (Cooper and Johnson 1992) with resulting steelhead termination (Eltrich 2007), the length of the period of time of the decline trend at Chambers Creek hatchery (as well as for the Skagit basin) could indicate a steadily declining productivity in the hatchery fish themselves that the shorter period of available hatchery data from that of wild in the Skagit basin can't depict. If that long-term declining productivity trend is conveyed to wild steelhead through spawning interactions and hybridization an eventual and ever increasing loss of productivity in the wild steelhead population would be a likely result.

The loss of productivity over time cannot be simply explained just by ocean conditions. Although the alternating ocean productivity patterns are often visible within the long steady decline in Skagit basin steelhead harvest since the 1970s (McMillan 2012; Figures 21-23), the length of the 40 year time period of that steady decline is not explainable by either the two common ocean productivity indices for West Coast salmon, the Oceanic Nino Index and the PDO Index.

One potential explanation for the long-term decline in Chambers Creek, as well as the Skagit River is the increased susceptibility to predation by predators that congregate off the mouths of where hatchery fish are outplanted and along the freshwater and early marine outmigration corridor. A recent southern British Columbia hatchery steelhead study at the Seymour River using acoustic tracking of smolt releases found that by barging smolts from the river to outside the estuary area for release resulted in higher survival (Balfry et al. 2011). Among considerations for estuary area losses were predator concentrations, pollution, and disease. Not further discussed was the potential that hatchery steelhead smolts themselves may attract increasing predator concentrations that are adapting to easy prey abundances at estuary locations. The protective inland marine waterways of Puget Sound and Georgia Strait may particularly attract predators with increasing hatchery smolt releases to sustain them. Also unconsidered were the potential effects of salmon and shell fish aquaculture operations in estuarine areas on both wild and hatchery steelhead during entry to the marine environment that could also be sources of pollution, parasites, and disease in the shared American/Canadian Salish Sea. Although the Canadian steelhead tracking study did not well answer why reduced marine survival reduction is geographically localized to Georgia Strait and Puget Sound, it does indicate location may be localized to estuaries. Thus hatchery programs can create their own environmental conditions that reduce their long-term production over time. Whatever the ultimate reasons may be, during periods of reduced steelhead productivity the Skagit and Chambers Creek steelhead data indicate that increasing hatchery plants can be counter-productive for both hatchery and wild steelhead in the Skagit (Figures 3, 8, 10, and 17; McMillan 2012; Figures 3, 6, 9, 14-19) and in other Puget Sound streams (McMillan 2012; Figures 24-43).

Can the elimination of hatchery plants result in a positive response to wild steelhead populations?

While the mechanism for the effects of hatchery outplants on wild populations may not always be clear, the potential experiment of reducing, changing, or eliminating hatchery production to measure how wild populations respond can occur. Several examples of this management action currently exist in the Columbia River basin. The Columbia basin provides the opportunity for particularly useful comparative wild steelhead trends related to hatchery releases due to a mix of rivers that includes some with a more direct means of tabulating steelhead returns through traps and dams and due to differing steelhead management strategies having occurred. Also, unlike Puget Sound, the Columbia basin has generally had overall good anadromous fish returns for a decade now. Nevertheless, while some wild steelhead populations have responded with increasing returns, others have continued to decline.

The Wind and Hood rivers are in close geographic proximity with their mouths entering the Columbia within 14 miles of each other (Bryant 1949; Parkhurst et al. 1950). Hatchery summer-run steelhead plants were discontinued on the former but continued on the latter as an apparently unrelated and accidental opportunity for experimental evaluation (McMillan 2012; Figures 69-76; Howell et al. 1985; Lucas and Nawa 1985; Cramer 1991; Scott and Gill 2006-b; Rawding 2012; Chilcote et al. 2011; ODFW 2011-a; Reagan 2011). The Hood River also has a winter-run steelhead population to provide other comparative values (Howell et al. 1985; Reagan 2011; Christie et al. 2012). Of particular note, as the wild summer-run steelhead population has continued to decline on the Hood River, that includes several studies documenting the negative hatchery implications for wild steelhead productivity (Araki 2008; Araki et al. 2007-a; Araki et al. 2007-b; Christie et al. 2011; Christie et al. 2012), since elimination of hatchery summer steelhead smolt plants at Wind River in 1998 the wild steelhead population has responded instead with an increasing recent trend found to be significantly better than the Hood River trend (Atlas 2011; McMillan 2012).

The Hood River winter-run steelhead population with continued hatchery steelhead plants is also in steady decline (Reagan 2011; Gerstenberger 2009; Christie et al. 2012) despite the Columbia basin apparently having otherwise good recent survival opportunity for anadromous fish. In both Hood River summer-run and winter-run steelhead cases, wild broodstock and domesticated broodstock (Blouin 2003; Araki 2008; Gerstenberger 2009) were used in the hatchery programs with reduced survival in the wild found for each and corresponding declining wild steelhead populations (Christie et al. 2012). Furthermore, a "carry-over" effect was found with lingering reduced productivity (Araki et al. 2009).

Further confirming the present Columbia basin positive anadromous fish opportunities for a wild summer-run steelhead population is the example of Asotin Creek in the Snake River basin where anadromous fish have to outmigrate and return through eight dams and reservoirs. Despite this, since hatchery steelhead smolt plants were discontinued in 1998, and a lower Asotin Creek weir has largely eliminated or minimized hatchery steelhead passage to the upstream spawning grounds since 2005, wild summer-run steelhead have responded with an upward trend that includes the most recent weir count that nearly equals the count of wild steelhead at a dam that was then on lower Asotin Creek in 1960 (McMillan 2012, Figures 65-68; Howell et al. 1985; Scott and Gill 2006-b; Crawford et al. 2012).

<u>The long-term performance of wild steelhead populations that have not had hatchery outplants</u>

If hatchery outplants do have deleterious effects on wild steelhead populations then are there examples in the Pacific Northwest where there have been no hatchery outplants and how have they fared with changing environmental conditions? There are two examples from the Oregon coast of winter-run wild steelhead trends without winter-run hatchery plants. The first is the Salmonberry River which is a tributary to the Nehalem River of Oregon's north coast. It has no known history of hatchery steelhead plants and is considered to have all wild spawning escapement (Chilcote et al. 2011; ODFW 2011-a) although it is possible some hatchery adults may stray into the Salmonberry from the Nehalem. Spawning surveys from 1973 to the present indicate a neutral trend over that time in peak redds counted per mile of stream (Ferguson 2009). Numbers of steelhead computed from the redd estimates are limited to the 1981-2000 period and indicate an increasing trend in that period of time (ODFW 2010). Sport catch data from 1979 to 2011 indicate a steep decline in the shift from a harvest fishery to that of wild catch and release (McMillan 2012, Figure 86). This would indicate that wild run-sizes may actually have declined despite the stable escapements. However, former peak annual steelhead harvests on the Salmonberry were generally low at 80-100 steelhead and would have altered the wild steelhead run-size trend very little over time. Wild escapements in the 1981-2000 period were 80-1,688 steelhead and wild run-sizes were 126-1,757 steelhead.

Another example is the Umpqua River that enters along the south-central Oregon Coast. The basin includes the North Fork and South Fork Umpqua and Smith River of the lowermost basin. The North Fork Umpqua has wild summer-run and winter-run steelhead. The North Fork Umpqua has had a minimal history of hatchery winter-run steelhead plants (sporadic fry plants 1947-65, smolt plants 1961-66 and very small numbers in 1999 and 2007-2011) (ODFW 2011-a). Counts of North Umpqua steelhead have occurred at Winchester Dam on the lower river since 1946 and differentiation of wild from hatchery and summer and winter runs have been determined or otherwise estimated (ODFW 2012-b). The North Fork Umpqua wild winter-run steelhead has been described as the most stable in Oregon (Kostow 1995) and has a relatively neutral trend pattern over the 64 year period of data depicted (ODFW 2012-b); (McMillan 2012, Figures 89 and 90). The sport catch has similarly remained at a neutral trend that only recently included catch and release regulations in portions of the stream (ODFW 1994; ODFW 2007; ODFW 2012-a). The average annual sport harvest of North Fork wild steelhead has been ~1,200 steelhead dating back to 1971. Of the four winter steelhead populations in Umpqua basin, only the NF was not declining at that time of 1995 evaluation (Kostow 1995).

From the perspective of comparison to the Skagit River and other greater Northwest regional steelhead streams, the North Fork Umpqua provides one example of a wild winter steelhead population that for about 65 years has remained remarkably stable and where the return pattern indeed apparently reflects the alterations in ocean productivity over time but with a neutral trend line sustained through the peaks and valleys of those alterations back and forth. Relatively low and evenly sustained harvest and lack of hatchery winter-run smolt plants has apparently allowed the natural steelhead productivity of the North Fork Umpqua to be expressed over a long period of time. This contrasts with two other winter-run steelhead tributaries in the Umpqua basin where hatchery plants have occurred and wild winter-run steelhead declines have resulted (Smith River and South Fork Umpqua; McMillan 2012; Figures 93 and 92). This is similar to the Siletz River basin and its tributary Drift Creek where winter-run hatchery programs have led to wild steelhead declines (McMillan 2012; Figures 87 and 88). Further to the north, the Salmonberry River (as previously discussed), again without hatchery releases, has a wild steelhead return pattern with a neutral trend of stability that replicates that of the North Fork Umpqua.

The long-term performance of Salish Sea wild steelhead populations

While examples exist from areas outside the Skagit of relatively stable wild steelhead populations, the question still remains of the relative influence of environmental conditions v. hatchery operations. One basin to examine is the Fraser River system which shares with the Skagit the commonality of occupying the Salish Sea and presumably similar ocean and freshwater environmental conditions over the same periods of time examined. Three watersheds we examined as a potential “reference” are from the Lower Fraser – the Coquihalla River, Silverhope Creek, and the Chilliwack/Vedder system.

In the lower Fraser River basin, snorkel surveys of the Coquihalla River summer-run steelhead have been ongoing since 1974 (McMillan 2012; Figures 117-120). Hatchery summer-run fry plants occurred in 1971 and 1981-1996, parr plants 1983-1988, yearling plants 1972-1979, and smolt plants 1980-86 and 1993-2003. There have been no hatchery plants since 2003. Hatchery steelhead have contributed proportionally less to the total sport catch than wild steelhead for most years from 1991 onward. The snorkel surveys are thought to represent mostly wild steelhead and the trend has been neutral over the long-term from 1961 to the present.

Silverhope Creek is just west of the Coquihalla where snorkel surveys have monitored the wild summer steelhead population since 1975 (pers. comm. Michael Willcox and Greg Wilson December 18, 2011) (McMillan 2012; Figures 121-122). Hatchery summer-run fry plants occurred from 1979 to 1985 but have since ceased. The steelhead return is all wild. Depending on the year, many steelhead may be missed in the snorkel surveys due to low flow conditions.

When flows are low steelhead are then thought to commonly over-summer in Silver Lake rather than in the stream above, with resulting low counts during snorkel surveys of the stream sections. The Silverhope wild summer-run steelhead population has been considered to be stable over time. The snorkel survey trend is near neutral with a slight decline and may be skewed toward decline due to missing steelhead in recent years of snorkel counts due to low flows and steelhead remaining in the lake. There is also by-catch of Silverhope bound steelhead in Fraser River fisheries that for some years is estimated to be as high as the escapement to the creek. Nevertheless, the Silverhope sport catch trend of wild steelhead has remained relatively neutral from 1962 to 2006 and supports the estimate of population stability.

The Chilliwack/Vedder River basin of the lower Fraser River has estimates of its wild steelhead escapements back to 1947 (Marshall et al. 1980). During the period of 1947 to 1975 the escapements were typically thought to be 2,500-15,000 steelhead, but from 1976 to 1979 the wild escapements quickly dropped to 400 wild steelhead. The lower Chilliwack/Vedder has had channel alterations, gravel removal, wing dam construction, and subsequent diking in the period from 1949 to 1964 followed by more extensive gravel removal in 1976 (Marshall et al. 1980). Logging and urbanization have since been identified as impacting fish habitat (Gow et al. 2011). Hatchery steelhead smolt plants began in 1973 but were not sustained until 1980 and have continued to the present using wild broodstock {integrated hatchery program} (MOE 2011; and Gow et al. 2011). Wild steelhead escapements since the mid 1980s have been estimated to average about 2,000 with run-size estimates of about 4,000 (McMillan 2012; Figures 123-127). Since 1966 the wild winter-run steelhead sport catch has had an increasing trend and hatchery sport catch has had a neutral trend since initial hatchery returns in the early 1980s. However, from 1984 to 2006 (latest data that was available) the wild and hatchery steelhead catch trends have had a slight decline. The long-term escapement trend has been one of decline, but it has been estimated as a neutral trend for both wild escapement and run sizes since the mid 1980s. Although the Chilliwack/Vedder wild winter-run steelhead population apparently experienced great decline in a transitional period of the 1960s and 1970s, perhaps related to habitat alteration in the lower basin and perhaps over-harvest, since that time the trend has been stable. The Chilliwack hatchery program differs greatly from those used in Puget Sound. Unlike the segregated programs commonly used in western Washington, the Chilliwack Hatchery is managed as a integrated program that captures a small number of wild steelhead each year. Marked smolts are not released from the hatchery but rather from a variety of locations 15-25 miles downstream of the hatchery site. The relative stability of the hatchery and wild populations may be a reflection of a considerably different approach to steelhead hatchery management that utilizes indigenous stocks and maintains a fairly small smolt release program.

The Lower Fraser steelhead data suggests that there is no similar continuous long-term steelhead decline as that found for the Skagit River and other Puget Sound steelhead populations in Washington. At the larger spatial (21 conservation areas) and temporal (35 years) scale of all British Columbia steelhead returns 3 had increasing trends, 13 were neutral, 4 were in decline, and 1 was in moderate decline (Ahrens 2004; McMillan 2012; Table 5).

With and without hatcheries – Examples from Vancouver Island British Columbia

Of a number of Vancouver Island streams reviewed three from northern Vancouver Island were chosen that had actual wild steelhead counts to compare to the Skagit River wild winter steelhead data. Two of the streams did not have significant hatchery outplants while one stream used a range of hatchery and habitat strategies. The Keogh, Heber, and Tsitika rivers were chosen as sharing the northern half of Vancouver Island but having different management strategies to determine if steelhead trends differed within them and for Skagit comparisons.

The Keogh River on northeast Vancouver Island is part of a long-term winter-run steelhead research effort that has occurred since 1976 (Slaney 1986; Ward and Slaney 1988). Wild and hatchery steelhead weir counts indicate a steeper decline for hatchery steelhead than for wild steelhead over the period of available counts from 1976 to 2008 (McMillan 2012, Figures 95-97; Slaney 1986; Ward and Slaney 1988). The Keogh steelhead research has used a wide range of methods and experiments to attempt to recover wild steelhead that include instream habitat projects, periods of nutrient enhancement (ceased in 2005), a wild broodstock hatchery program that has ceased (1979-1990, and 2002 fry or smolt releases), and most recently a living gene bank (LGB) hatchery program of using wild smolts kept captive to spawning maturity for egg taking with subsequent smolt releases that has also ceased (2003-2005) (McMillan 2012). Adult returns from the LGB program indicated poor survival results as evidenced by smolts from 2005 having returned at 2.6% for wild fish and only 0.5% for LGB releases and with further concerns that LGB-derived hatchery fry and parr, as well as residualized hatchery fish after release, may have affected wild smolt yield (McCubbing and Ward 2008).

Through all these experimental approaches, since the latter 1980s, the Keogh wild steelhead have remained at low population levels and of great conservation concern. Even in quite close geographic proximity there are steelhead streams with moderately to significantly different wild steelhead trends than that of the Keogh River on Vancouver Island, but there are also those streams with similar trends. In the case of the Keogh it may be that the research activities themselves have led to steelhead declines significantly greater than what may naturally have occurred.

The Tsitika River is also on northeastern Vancouver Island. It's summer-run of steelhead has been monitored by snorkel surveys from 1976 to the present (McMillan 2012, Figure 103). The Tsitika had periods of hatchery fry plants between 1978 and 1989 but no smolt plants. It's wild summer steelhead returns have had a slight decline that is recently increasing, very different from that of the steelhead trend at the Keogh River for the same period of comparison.

On the west side of Vancouver Island almost opposite from the Campbell/Quinsam basin is the Heber River that is tributary to the Gold River. It has a population of wild summer-run steelhead unaffected by hatchery steelhead releases except for one fry plant in 1983. It has had long-term snorkel surveys dating from 1975 to the present (McMillan 2012, Figures 109-111) with a neutral trend of wild steelhead stability and with an increasing wild sport catch trend (data provided in 2011 and 2012 by pers. comm. Mike McCulloch of the BC Ministry of Forests, Lands and Natural Resource Operations).

Of these three northern Vancouver Island examples, only the Keogh River steelhead population displays a steelhead trend similar to that of the Skagit River. Although these three Vancouver Island streams have the best comparative data related to actual counts of their steelhead populations, there are catch data and less complete snorkel survey data for other east side Vancouver Island steelhead streams that were examined. Some of those have wild steelhead trends more similar to the Keogh while others have trends more similar to the Tsitika and Heber. In other words, the trends are variable and not consistently the same despite roughly similar geographic relationships, or even sometimes in quite close geographic proximity.

Whatever ocean conditions may have been, the wild steelhead outcomes have differed among these streams, for the other east side Vancouver Island streams examined (McMillan, 2012), and as Ahrens (2004) similarly found for greater British Columbia over the same comparative time periods.

12.0 Synthesis of Chapters

Many factors contribute collectively to Puget Sound steelhead population declines by altering survival and productivity (Hard 2007). The Puget Sound Steelhead Technical Recovery Team (TRT) recently indentified the following factors of decline:

- the present or threatened destruction, modification, or curtailment of its habitat or range
- overutilization for commercial, recreational, scientific, or educational purposes
- disease or predation
- the inadequacy of existing regulatory mechanisms
- other natural or manmade factors affecting continued existence

The focus of this study was on a single manmade factor best described as a "hatchery" effect. At a finer level of examination, this study examined the ecological and genetic effect of interactions between hatchery and natural-origin steelhead in the Skagit watershed. The lifestage approach used in this study allowed for evaluation at both juvenile and adult lifestage sub-periods. These results, when combined with an attempt to quantify the level of genetic introgression and a statistical analysis of the impacts of the hatchery steelhead smolt outplants on steelhead population trends, were all used as input to our meta-analysis (see Figure 1). The results of the meta-analysis form a "weight of scientific evidence" that will be used by fish managers to form and implement hatchery based recovery actions as part of a more comprehensive recovery plan for Skagit natural-origin steelhead.

The implications of ecological and genetic interactions between hatchery and natural-origin steelhead have been identified as an important factor in the overall viability of natural-origin populations by other researchers (Araki 2008, Brown et al. 2012, Christie et al. 2011, Kostow 2009, Seamons et al. 2010, Hard 2007). The TRT employs four parameters for measuring the viability of steelhead populations (Ford 2010) as defined by McElhaney 2000. Our study findings will be used, where applicable, to determine how these parameters of viability are affected relative to Skagit River natural-origin steelhead.

This synthesis section consists of four parts; a meta-analysis summary using the lifestage approach used throughout this report, a statistical evaluation using population data, TRT population viability and lastly a fish management alternatives matrix followed by a final recommendation.

12.1 Juvenile Lifestage

During the juvenile lifestage there were two differing categories of interaction occurring within the Skagit basin. The first results from the annual release of hatchery smolts. Beginning at time of release, hatchery smolt begin interacting at an ecological level with wild origin conspecifics during the freshwater and early marine outmigration period. The second category initiates from hatchery fish straying and spawning outside the hatchery creating hybrid and naturally spawned hatchery-origin juveniles. This category has both genetic and ecological implications.

There is a clear overlap in freshwater outmigration timing and habitat space used by natural-origin and hatchery released smolts. During the 2-4 week freshwater outmigration period exhibited by hatchery smolts space and food resources are shared with their natural-origin counterparts. Although the effect of this hatchery release pattern on natural-origin smolts remains unclear it is undoubtedly an opportunity for competitive interactions to occur for both habitat and food resources during the freshwater, estuarine and early marine stages of emigration. These interactions can affect the growth potential and survival of natural-origin smolts.

Similar overlaps in timing were detected during the early marine migratory period of both groups. However, it appears that the marine pathway chosen by hatchery and natural-origin smolts may differ somewhat which would effectively reduce the relative potential for interactions while moving through Whidbey basin and northern Puget Sound.

Other studies have demonstrated that the ecological effects of hatchery programs may significantly reduce wild population productivity and abundance even where genetic risks do not occur (Kostow et al. 2003; Kostow et al. 2006). The number of hatchery smolts released at the Skagit each year can and does vary. During some years it appears that the release size can greatly outnumber the natural-origin population size elevating potential levels of competition for food resources and space. One of the HSRG recommendations on this topic prescribed the smallest possible release to meet the management goal of the program while avoiding the potential for ecological interactions with natural populations. Ideally, from an ecological standpoint the hatchery production level should be of a smaller magnitude than that produced by their natural-origin counterparts. Another benefit to a smaller release size is possible avoidance of predator attraction during the peak of natural-origin outmigration.

If natural-origin steelhead recovery is a high priority to fish managers, a reduction in the size of the Marblemount hatchery program may be warranted to reduce ecological interactions with natural-origin juveniles during the freshwater and marine lifestages. Ecological interactions between these two groups are occurring when both are at their highest densities. These interactions could potentially affect growth and survival of natural-origin outmigrants due to competition for food or space. In addition, it is also unclear whether known predators, such as bull trout, might react differently to the presence/absence or magnitude or release timing of hatchery-origin migrants.

Negative ecological interactions between these two groups could largely be eliminated by separating the outmigration timing. To reduce interactions with natural-origin smolt a delayed hatchery release timing should be considered to avoid the natural-origin smolt outmigration peak density period. Such an action would greatly reduce the overlap in time and space during the freshwater and marine lifestage of these conspecifics. As a result of a delayed hatchery release, natural-origin migrants would presumably have less competition for space and food resources that might be critical to their survival during the freshwater and early marine lifestage.

The second category of interaction stems from hatchery fish straying and spawning outside the hatchery. At the juvenile lifestage interactions of this type is expressed both genetically and ecologically. The ubiquitous presence of hybrid and natural spawned hatchery juveniles was established using two different methods of analysis. However, it should be understood that the best available tools for measuring introgressive hybridization continue to lack the ability to quantify introgression with high levels of confidence. The new method developed for this use, as described in section 10 of this report, while perhaps an improvement, continues to fall short of the desired scientific rigor. While some caution should be exercised in using these microsatellite-based results, both methods used in this report revealed the presence of both hybrid and naturally spawned hatchery juvenile throughout the watershed including large mainstem reaches, major sub-basins such as the Sauk, Suiattle and Cascade rivers as well as in smaller tributaries regardless of distance from the hatchery source. It should be noted that to improve upon the quantification of introgression, Warheit recommends repeating the same microsatellite based method described in this report using single nucleotide polymorphism (SNP) to more accurately quantify hatchery introgression among the steelhead populations within the Skagit River basin.

The hybrid densities found in the Skagit collection areas ranged from 4% in the Sauk collection area to 26% in Finney Creek (Table 42). The two collection areas with the highest hybrid densities were both from middle Skagit River tributaries; Finney and Grandy creeks. This finding also coincides with tributaries with the earliest natural-origin spawn timing in the basin. The high hybrid densities, strongest introgression signal, observed in these tributaries may be the result of where the longest spawn time overlap exists between stray hatchery and early spawning natural steelhead. Similarly, naturally spawned hatchery juvenile densities varied by location (Table 42). Together, the hybrid and naturally spawned hatchery juveniles densities levels ranged from 8-36% of the steelhead juveniles tested. The results derived from these two methods were very similar.

Table 42. Comparison of hybrid and natural spawned hatchery juvenile densities derived from two methods of genetic ancestry evaluation.

Evaluation Method	Juvenile Hybrid Density Range	Naturally Spawned Hatchery Juvenile Density Range
Structure 2.1 (Section 9)	4-26%	2-10.6%
Warheit (Section 10)	6-23%	2-11%

There are multiple ecological and genetic implications associated with the presence of hatchery-origin steelhead juveniles in the natural production zone and adults spawning naturally outside of the hatchery. Many of these ecological and genetic risks were described by Kostow (2009) and Seamons (2010). Kostow identified a number of factors that singularly or in combination are capable of compromising the productivity and genetic integrity of natural-origin steelhead populations. She found that ecological risks occur when the presence of hatchery fish affects how natural-origin fish interact with their environment or with other species. Some of the more important contributing factors were identified as; relative abundance of hatchery and natural-origin fish in the natural production areas, hatchery programs that increase density-dependent mortality, residual hatchery-origin fish, and some physical advantages that hatchery fish can have over wild fish. All of these factors are thought to be at play in the Skagit. It appears that in most years the number of hatchery smolt released outnumber that derived from natural-origin production and likely compete for space and food resources during the freshwater and early marine lifestage. Our work has also shown the presence of both hybrid and naturally spawned hatchery juveniles throughout the basin that compete for space and food resources in natural production areas. Just as Kostow described in her work, the naturally spawned hatchery and hybrid juveniles in the Skagit may well have a physical advantage over juveniles of natural-origin. It is expected that the earlier spawn and fry emergence timing of hatchery influenced juveniles allow for these fish to occupy habitat before the later emerging natural-origin fry. Any growth by the early emergent hatchery influenced fry likely provides a further physical advantage over the later emerging natural-origin fry creating another ecological interaction that could effect the growth and survival of natural-origin juveniles throughout the basin. This ecological outcome is further supported by other researchers, such as Seamons, where he suggests that hatchery ancestry offspring of earlier spawning hatchery steelhead may emerge from the gravel (Mackey et al. 2001) and obtain feeding territories before wild ancestry fish. Earlier emerging fish will be larger (because they had time to grow) when the offspring of later spawning fish emerge (Einum and Fleming 2000), and larger size provides fitness advantages (Abbott et al. 1985; Rhodes and Quinn 1998).

Seamons studied the strategy of segregation by divergent lifehistory in steelhead where hatchery-origin fish were selected to spawn months earlier than the indigenous wild population. This same strategy has been in use for 60-years in the Skagit watershed with steelhead. After three generations, Seamons found that the genetic makeup of the indigenous population he studied had shifted with the proportion of natural-origin ancestry smolts and adults declining by 10-20%. In addition, he found that up to 80% of the naturally produced steelhead were hatchery-natural-origin hybrids. Importantly, he also discovered that the hybrid proportions in both smolts and adults shifted quickly relative to the number of naturally spawning hatchery fish present.

The initial stage of genetic introgression thought to take place in the Skagit results from hatchery and natural-origin steelhead reproducing together. Progeny resulting from this mating combination are considered an F1 hybrid because the juveniles result from two distinctly different parent types. If the F1 progeny survive to adulthood another stage of introgression can occur that involves three other predictable mating combinations. These combinations include the following mating types; F1 progeny x F1 progeny, F1 progeny x hatchery-origin adult and F1 progeny x natural-origin adult. Presuming there is some survival to adulthood from each of these mating types, over multiple generations it is reasonable to assume that the population would be comprised of some individuals displaying a full range of hatchery/natural-origin genetic ancestry. From each of the collection areas studied on the Skagit we found examples of individual steelhead displaying the full range hybrid ancestry at both the juvenile and adult level (see appendix 14.3). The proportion of hatchery ancestry measured from individual fish ranged from 20% to over 90% in nearly all areas (collections). The distance from the hatchery source did not appear to influence the hybrid density observed in juveniles or adults. Based on our data, we observed and speculate that hatchery or hybrid adults stray and spawn throughout the watershed. Similarly, the homogeneous genetic makeup of the Skagit natural-origin steelhead also strongly suggests some level of adult straying is probable resulting in a blending of natural-origin genetic materials throughout the basin and perhaps into adjoining basins as well. Because both natural and hatchery-origin steelhead found in the Skagit watershed appear to stray within the basin it seems possible that both hatchery-origin and hybrid adults may both be transferring hatchery ancestry throughout the basin by way of the same straying mechanism.

The genetic based analyzes presented in several chapters of this report suggest that hatchery fish, or their offspring, are spawning with wild fish in the Skagit basin. This would result in lowered population productivity rates for wild steelhead, given that hatchery fish have substantially lower marine survival rates than wild fish.

There have been numerous studies dating from the 1970s to the present indicating that hatchery steelhead do not perform well in the wild and can have subsequent negative effects on wild steelhead (Reisenbichler and McIntyre 1977; Chilcote et al. 1986; Araki 2007-a; Araki 2007-b; Araki 2008; Araki et al. 2009; Seamons et al. 2012; Byrne and Copeland 2012; and Christie et al. 2012) and this can occur in a very short time (Christie et al. 2011).

Numerous researchers have found that non-native broodstock like that used at the Marblemount hatchery often have lower reproductive success than their natural-origin counterparts due to a lack of local adaption and from the prolonged effects of domestication (Kostow et al. 2003; Araki et al. 2007a; Araki et al. 2008; Chilcote et al. 2011; Fraser et al. 2011). Reproductive success of hatchery steelhead from the Marblemount facility has shown a consistent downward trend over the past two decades (see section 11). Because of this reduction in reproductive success interbreeding between hatchery and natural-origin steelhead in the Skagit basin may contribute to long term negative fitness consequences for the wild population.

From a population standpoint there are several ecological implications to the presence and density of hatchery and hybrid juveniles shown to be present throughout the Skagit watershed.

- Hybridization reduces the reproductive potential
- Hybridization comes at the cost of reduced productivity
- The return and spawn timing of hybrids surviving to adulthood may create additional reproductive overlap with natural-origin adults. Seamons found that once hybridization occurs the temporal separation of spawn timing for these individuals may be compromised. Seamons suggests that the migration and spawn timing of hybrid steelhead would be expected to overlap with the timing of both populations. His results showed that putative steelhead hybrids tended to spawn later than expected and closer to the spawning season of the indigenous population.

At the juvenile lifestage the segregation of the hatchery and natural-origin populations fall short of desired outcome from both an ecological and genetic standpoint.

12.2 Adult Lifestage

The early migration and spawn timing of Chambers Creek origin hatchery fish used at the Marblemount hatchery were believed to effectively prevent interbreeding with natural-origin steelhead found in the Skagit basin. It has also been assumed that hatchery adults, upon return, would exhibit high levels of spawn site fidelity and return to the hatchery instead of straying elsewhere in the basin. Instead, our data finds that significant proportions of both juveniles and adults are either hatchery-wild hybrids or natural spawned hatchery-origin fish. The two genetic ancestry methods used in this study demonstrate that naturally spawned adults of hatchery-origin are present at varying levels within the basin and densities in natural production areas range up to 10% depending on the location (Table 43). Adults of with hybrid ancestry were also found to occur throughout the basin. The strongest signals of hatchery introgression in adults were found to be from Finney Creek, Sauk River, and upper Skagit River. These results suggest that some level of presumptive hybrid progeny are surviving to adulthood. In addition, it appears that some of the progeny resulting from adult hatchery crosses that spawned naturally are also surviving to adulthood.

Table 43. Comparison of hybrid and natural spawned hatchery adult densities derived from two different methods of genetic ancestry evaluation.

Evaluation Method	Adult Hybrid Density Range	Naturally Spawned Hatchery Adult Density Range
Structure 2.1 (Section 9)	15.4 - 34%	0 - 4.9%
Warheit (Section 10)	5 - 20%	2 - 10%

It remains unclear how the juvenile and adult hybrid densities may change over the course of multiple reproductive generations. Although we suggest that there are many factors in force influencing the density of putative hybrids in the natural population, this study presents a set of genetic data from Sauk River adults that spans 30-years (see section 8). This dataset, while only providing a cursory assessment, suggests that the scope of Skagit hybridization may have remained fairly static over this three decade period.

Evidence presented in earlier sections of this report found that marked hatchery adults are present in the Skagit after February in both spawned and unspawned condition. These fish exhibit several undesired outcomes for a segregated hatchery adult; spawning outside the hatchery, demonstrating a return timing that overlaps that of the current natural-origin conspecifics and using spawning habitat in regions of the basin utilized by natural-origin steelhead.

Others researchers have shown that much of the variation in migration and spawn timing in salmonid fishes is due to additive genetic variation (Carlson and Seamons 2008). They suggest that migration and spawn timing of steelhead hybrids would be expected to overlap with the timing of both populations. Little is known about whether there is a genetic component to return timing in salmonids. However, available research suggests that for steelhead, once hybridization occurs the temporal separation of spawn timing for these individuals may be further compromised. Seamons suggests that the migration and spawn timing of hybrid steelhead would be expected to overlap with the timing of both populations. His results showed that putative steelhead hybrids tended to spawn later than expected and closer to the spawning period of the indigenous population. This same research and ours found that late returning non-hybrid hatchery-origin adults, especially males, on the Skagit were found to stay in fresh water for many months. Other studies found that hatchery males in particular are capable of remaining in fresh water until natural-origin females arrive and mate with wild fish throughout the wild spawning season, thus producing offspring with relatively late return timing (Leider et al. 1984; Seamons et al. 2004). Late returning hatchery strays may mate with natural-origin adults, especially those that spawn early, producing F1 offspring with relatively late return timing. It was further suggested that although spawn timing may provide some reproductive isolation between fish of hatchery and wild descent, any hybrids would spawn later and with larger temporal overlap with wild fish may increase the incidence and level of introgression in the long term. This may be the mechanism that explains why on the Skagit the largest overlap in spawn timing occurs in the middle Skagit reach, especially in the tributaries where some of the earliest natural-origin spawning takes place.

There is evidence that the historical run-timing of natural-origin Skagit steelhead included a greater number of earlier run-timed fish that have largely disappeared. In March of 1901 hatchery racks for steelhead egg taking were put in Finney and Grandy creeks but met with minimal success (Ravenel 1902). It was determined the racking was too late and the major run of steelhead had been in January. In early December, 1902 racking operations commenced at Finney and Grandy creeks and better success was achieved (Titcomb 1904). During Sauk River racking in 1907 steelhead spawning began in early February (Riseland 1907). During 1954-55 return year the sport catch data for the Skagit River was 68% in December-February and 32% in March and in 1955-56 it was 56% in December-February and 44% in March-April (WDG 1956 and 1957). This short period of record was just prior to initiation of the Skagit steelhead hatchery program which began with smolt plants in 1960 (Royal 1972). This evidence strongly suggests that historically wild steelhead run-timing to the Skagit River would have directly overlapped with that of Chambers Creek hatchery stock as did entry to tributary spawning locations.

When present these fish would have had greater opportunity to genetically interact during the first few decades of the segregated hatchery program when their run-timing coincided with these early run-time fish. It is possible that the elevated levels of hybridization observed in Finney and Grandy creeks may represent remnant examples from that earlier time period.

While it appears that natural-origin adults are physically prevented from reproducing with hatchery-origin adults inside the hatchery facility, our ancestry data suggests that a weakness of the segregated program used on the Skagit is that hatchery fish can not be physically, temporally or behaviorally prevented from straying and spawning naturally with other hatchery strays or natural-origin adults. Although this core principle of segregated programs is critical to protect the genetic integrity of the natural-origin steelhead it does not appear to be working as effectively as desired on the Skagit or in some other systems studied (Kostow 2009, Seamons 2010). The observed Skagit outcome is somewhat predictable when the contributing factors are considered collectively.

First, hatchery managers have no effective tool to prevent hatchery-origin steelhead from choosing to spawn outside of the hatchery. Secondly, we have shown that both hatchery and natural-origin adults exhibit a level of straying. For hatchery strays this leads to reproductive alternatives that include other hatchery strays, natural-origin or hybrid adults that are all found throughout the basin in varied densities. The final factor involves temporal separation of spawn timing between hatchery and natural-origin populations. The data presented from this study provide evidence showing that there are overlaps in spawn timing that exist in the Skagit watershed. The spawn time overlap may vary by return year, hatchery individual and the presence of hybrids as described by Seamons.

Our data further suggests that steelhead of both natural and hatchery-origin stray within the watershed and perhaps into adjacent basins. Natural-origin adult straying doesn't appear to be limited to within the Skagit basin. It appears there may be a geographical element to straying amongst local populations. Recent comparisons of Skagit, Nooksack and Stillaguamish natural-origin collections revealed that all three populations are quite similar from a genetic standpoint. Similarly, the genetic makeup was very similar for three natural-origin steelhead stocks from neighboring Olympic Peninsula river basins all emptying into the Pacific within approximately 50-miles of each other. It appears that there maybe some instances where natural-origin straying appears to extend to adjacent watersheds. The suspected genetic exchange occurring between natural-origin steelhead throughout the Skagit watershed may be responsible for the homogenized genetic signature. This genetic signature is also present in watersheds adjacent to the Skagit basin. If true, these findings suggest that some and perhaps all steelhead stocks have what might be an evolutionary tendency to stray. If straying is ubiquitous for the anadromous form of this species it becomes problematic to continue using this type of fish for a segregated hatchery program that is premised on the limiting interactions between hatchery and natural populations.

If fish managers decide that it is important to reduce the levels of hybridization observed in the Skagit it would logically stem from some level of reduction in the size of the hatchery release since segregation by life history differences (spawn time) have failed to prevent undesired genetic and ecological interactions.

12.3 Hatchery-Origin

The longterm manifestation of physical segregation is displayed by a comparison of the ancestry data from the Marblemount hatchery and natural-origin populations. The ancestry of individual fish returning to the Marblemount hatchery (Chambers stock) demonstrates that genetic exchange from the natural-origin population is very limited as would be expected with a segregated hatchery program where adults of natural-origin can be physically excluded from entering the hatchery. From a hatchery standpoint physical segregation appears to be producing the desired outcome resulting in only 0.6% of hatchery adults exhibiting levels of natural-origin ancestry (>20%) that would be expected from hybridization with natural-origin fish. The remaining 99.4% of the hatchery adults examined contained lower levels of natural-origin ancestry (<20%). The putative hybrid density in the hatchery population is quite low as compared with hybrid densities levels found in Skagit natural-origin adults and juveniles. After more than 60-years of Chambers-based hatchery production at the Marblemount hatchery the infusion of natural-origin ancestry remains extremely low in the hatchery stock. Sound hatchery practices exercised during this period appear to have consistently prevented natural-origin adults from entering the hatchery facility.

While it appears that natural-origin adults are physically prevented from reproducing with hatchery-origin adults in the hatchery, our ancestry data suggests that a weakness of the segregated program used in the Skagit basin is that hatchery fish can not be physically, temporally or behaviorally prevented from straying and spawning naturally with other hatchery strays, hybrids or natural-origin adults. Although this core principle of a segregated program is critical to protect the genetic integrity of the natural-origin steelhead it does not appear to be working as effectively as desired on the Skagit or in some other systems studied (Kostow 2009 Seamons 2010). The observed Skagit outcome is somewhat predictable given the nature of the contributing factors described in this report. It should be further understood that fish managers have no effective tool that can be used to prevent hatchery-origin steelhead from spawning outside of the hatchery.

It also appears, based on scale interpretation, that Skagit hatchery strays are capable of repeat spawning outside of the hatchery. While this occurrence is assumed to be small in number, multiple reproductive cycles by hatchery strays further complicates the potential amount of genetic and ecological interaction with the natural-origin population.

Stray hatchery adults, rather than return to the Marblemount hatchery as desired, instead opt to spawn in areas designated exclusively for natural-origin production. The exact degree to which hatchery-origin steelhead stray and residualize in the Skagit remains unclear but appears to exceed the 5% threshold established by HSRG (see tables 28, 35, 37). For hatchery strays this leads to reproduction opportunities outside the hatchery that include mating with other hatchery strays, natural-origin or hybrid adults all found throughout the basin in varied densities. However, hatchery stray levels likely vary annually depending on factors such as number of smolt released, smolt to adult survival and freshwater flow conditions during adult upstream migration. Finally, given that both hatchery and natural-origin steelhead returning to the Skagit basin demonstrate a tendency to stray it draws to question the efficacy of using this species in a segregated program.

Reduced hatchery fitness is another factor that can be additive with regard to genetic interactions. It has been well documented that there is lost spawning effort when wild fish spawn with hatchery fish, and reduced fitness when wild fish hybridize with hatchery fish that have substantially reduced marine survival rates. We also know that there is a cumulative effect of reduced fitness in steelhead hatchery operations as has been well documented (Araki 2007, 2008). In section 11 of this report we suggest that long-term and continuous decline in productivity seen with Marblemount steelhead may be conveyed to wild steelhead through spawning interactions and hybridization causing an eventual and ever increasing loss of productivity in the wild steelhead population (see figure 45).

Possible changes in future hatchery operations at the Marblemount facility should attempt to avoid or minimize interactions with natural-origin steelhead by altering smolt release timing, reducing smolt release size, or suspending the smolt release program altogether.

12.4 Population Level Analysis

To identify the potential long-term impacts of segregated hatchery programs, with respect to other factors, on steelhead returns in the Skagit River a multiple-step approach was taken. A complete review of section 11 of this report is needed to fully understand the complexity of the overall analysis approach. The three key steps of the analysis were:

- A linear regression analysis was used to examine the relationship of three independent variables – Skagit hatchery smolt release numbers, surface sea temperatures, and Skagit peak flows – on the annual abundance of returning adult steelhead to the Skagit River.

- A multiple regression model on the combined effects of a number of independent variables, including hatchery smolt release numbers, on wild steelhead returns in the Skagit (the dependent variable). This was done to control for possible intercorrelations between hatchery release numbers and variables related to shifting ocean conditions and hydrological conditions. We included a number of independent variables, including annual hatchery release numbers (15-year moving total), the PDO index, annual peak flows, annual low flows, and mean annual flows, in this analysis.

- A regional analysis on the effects of smolt releases on the productivity of wild steelhead productivity among a number of major Puget Sound river basins.

We found that there is a combined influence of three independent variables - Skagit hatchery smolt release numbers, surface sea temperatures in the North Pacific, and peak flows in the Skagit – on native Skagit steelhead adult returns. This analysis also allowed us to identify the relative effect of hatchery smolt release numbers on adult native returns while controlling for the influence of the other two independent variables. This analysis found that all three independent variables (smolt release numbers, surface sea temperatures, and Skagit peak flows) contributed significantly to native steelhead returns. Moreover, inter-correlations among the three independent variables were found to be very weak, meaning that the statistical effects of each variable were not being influenced by the other variables. Consequently, hatchery smolt releases were found to have a highly significant and negative effect on native steelhead returns in the Skagit that was independent of long-term trends in marine and freshwater conditions.

The inverse relationship between hatchery smolt releases and wild steelhead returns that is observed when examining the time-series plots of these two variables over the past 30 years is potentially the result of negative biological interactions occurring between hatchery and wild steelhead, including increased juvenile competition, increased susceptibility to predation by predators such as bull trout, lost spawning effort when wild fish spawn with hatchery fish, and reduced fitness when wild fish hybridize with hatchery fish that have substantially reduced marine survival rates.

While hatchery steelhead in the Skagit have had an numerical advantage in the freshwater environment over the past 15 years due to the release of large numbers of hatchery fish, wild steelhead have the distinct advantage of being able to produce a far greater number of spawners for the same number of smolts (higher productivity). Wild steelhead smolts have produced between 2 and 20 times more adults than hatchery smolts since 1997 (see Figure 46). The very low SAR rates observed in hatchery steelhead in other Puget Sound rivers during this same period (WDFW 2008) suggests that Chambers Creek hatchery stocks are not well adapted to the poor ocean conditions that have occurred in recent years.

Both wild steelhead and hatchery steelhead smolts in the Skagit have an average length of 180 mm (WDFW smolt trap data), suggesting that the difference in marine survival cannot be explained by smolt size alone. There are several possible explanations for the higher survival rates of wild over hatchery steelhead in the marine environment: 1) wild have higher survival rates while migrating through the Puget Sound, and are less susceptible to predation; 2) wild steelhead migrate to more productive areas of the north Pacific than hatchery fish; and 3) wild steelhead are better adapted to local conditions in the marine environment, and therefore have a greater ecological fitness to marine conditions than hatchery fish. While the specific reason for this difference in marine survival remains unclear, our analysis suggest that wild fish have a selective advantage over hatchery fish in terms of their population productivity as a result of substantially greater marine survival rates.

The regional analysis on the effects of hatchery smolt releases on native steelhead productivity among Puget Sound watersheds suggest that hatchery releases have had a long-term negative impact on steelhead population growth rates (see Figure 54). Although differences in habitat quality are likely a major factor explaining the variability in population growth rates (i.e., productivity) among Puget Sound watersheds, the results of this analysis suggest that hatchery practices also have a significant influence on productivity patterns of wild steelhead in this region. This further corroborates our finding that hatchery steelhead releases have had a long-term impact on native steelhead returns to the Skagit River basin.

The likelihood of a cause-and-effect relationship between hatchery releases and wild steelhead populations is further supported by the findings of the genetic analysis conducted for this report, which suggests that juvenile steelhead with various levels of hatchery-origin ancestry are present throughout the rearing areas for wild steelhead in the Skagit basin. The widespread presence of juveniles with hatchery ancestry suggests that ecological impacts on wild juvenile fish, including that of competition for habitat and food, are likely. The genetic analysis presented in other chapters of this report also suggest that hatchery fish, or their offspring, are spawning with wild fish in the Skagit basin. This would result in lowered population productivity rates for wild steelhead, given that hatchery fish have substantially lower marine survival rates than wild fish.

The long-term decline in SAR rates for hatchery steelhead is not unique to the Skagit, but has also been observed in other Puget Sound rivers including the Puyallup and Elwha (WDFW 2008). The average SAR index for winter-run (Chambers Creek origin) hatchery steelhead in the Puget Sound has declined from a peak of 7.0% in the mid 1980s to less then 0.4% through the 2000s. The SAR rates observed for Puget Sound steelhead hatchery stocks have remained the lowest among artificial steelhead production programs in the State of Washington since the mid 1990s (Scott and Gill 2008).

These marine survival rates are very low compared to average survival rates measured for wild steelhead in long term studies conducted in the Georgia Basin. With the exception of the Snow Creek monitoring study on the Hood Canal, there have been no long-term monitoring efforts for wild steelhead freshwater survival in the Puget Sound. The Snow Creek steelhead population does not correlate well with long term abundance trends for wild steelhead in the Skagit. However, there is a high degree of correlation between wild steelhead populations in the Skagit and Keogh River in the northern Georgia Basin (to be discussed later), with the Keogh River providing a high quality long-term dataset for wild steelhead in British Columbia (McCubbing and Ward 2008).

There are several examples in section 11 of this report that describe how wild populations have been shown to respond to the elimination of hatchery smolt releases. One such example occurred on the Wind and Hood rivers which are in close geographic proximity with their mouths entering the Columbia within 14 miles of each other. Hatchery summer-run steelhead plants were discontinued on the former but continued on the latter creating an unrelated opportunity for experimental evaluation (see section 11). The Hood River also has a wild winter-run steelhead population to provide other comparative values. Since this time the wild summer-run steelhead population has continued to decline on the Hood River, while on the nearby Wind River, after the elimination of hatchery summer steelhead smolt plants the wild steelhead population has responded instead with an increasing recent population trend. During this same time period, the Hood River winter-run steelhead population is also in steady decline with continued hatchery steelhead plants fish.

12.5 Viability Parameters

The implications of ecological and genetic interactions between hatchery and natural-origin steelhead have been identified as important factors in the overall viability of natural-origin populations by other researchers (Araki 2008, Brown et al. 2012, Christie, et al. 2011, Hard et al. 2012, Kostow 2009, Seamons et al 2012). The Puget Sound TRT employs four parameters for measuring the viability of steelhead populations (Ford 2010) as defined by McElhaney 2000. Using our Skagit study findings we evaluate how these "parameters of viability" are affected by the ecological and genetic interactions we observed between hatchery and natural-origin steelhead of the Skagit basin. For each of the four criteria we consider how the viability of the natural-origin population would be affected at the juvenile and adult lifestage. We also felt it would be useful to evaluate the viability of the hatchery population using the same criteria.

Our evaluation suggests that the Skagit's natural-origin population viability is negatively affected by interactions with hatchery-origin steelhead for three of the four criteria as shown in Table 44. The spatial structure of the natural-origin steelhead population appears to be the only viability criteria unaffected by interactions with hatchery-origin steelhead.

Table 44. Effect of ecological and genetic interaction factors on Skagit steelhead viability (based on Puget Sound TRT VSP criteria)

Puget Sound Technical Review Team VSP Criteria	Natural-Origin Juvenile Lifestage	Natural-Origin Adult Lifestage	Skagit Hatchery-Origin
Population Size (Abundance)	**Reduction** in population during multiple lifestages and on a spatial level due to presence and density of hybrid and natural spawn hatchery juveniles. Hatchery smolt interactions in freshwater and early marine lifestages may cause additional density dependent reductions in abundance.	**Spatial Reduction** in population due to presence and density of hybrid and size of smolt release.	**No effect -** Physical barrier at hatchery minimizes natural-origin hybridization opportunities. Hatchery returns are reduced due to straying outside hatchery.

Puget Sound Technical Review Team VSP Criteria	Natural-Origin Juvenile Lifestage	Natural-Origin Adult Lifestage	Skagit Hatchery-Origin
Population Growth Rate (Productivity/Survival)	**Reduced** productivity caused by lowered survival of hybrid juveniles and ecological factors related to hybrid and hatchery-origin habitat occupancy interactions causing, reduced survival and growth potential.	**Reduced** productivity/survival resulting from hybridization and associated reduction in potential spawner pool.	**Reduced** -Smolt to adult survival rates have consistently fallen for three decades to extremely low levels despite reproductive isolation from natural-origin population in the hatchery.
Spatial Structure	**No Change** -Genetic similarities between spatial collections suggest natural-origin juveniles are the result of unlimited connectivity and genetic exchange between areas. Genetic and ecological interactions with hatchery-origin population have not affected the spatial structure of natural-origin population	**Unchanged -** spawning location infidelity (straying) in both natural/hatchery-origin and hybrids have resulted in genetic connectivity throughout basin with natural-origin population and the spread of hatchery-origin genetics via. hybridization	**Expanded -** The spatial distribution of hatchery-origin population has expanded from the desired isolation at the hatchery location to all spatial areas studied within the basin
Diversity (Phenotypic & Genetic	**Impacted -** Ancestry data show genetic variation resulting from hybridization among individuals in natural rearing areas throughout basin. The density of juveniles with genetic variation varies spatially.	**Impacted -** Hybrids are surviving to adulthood. Ancestry data shows genetic variation resulting from hybridization among adult individuals in natural rearing areas throughout basin. The density of adults with genetic variation varies spatially.	**Exceeds Threshold -** The level of hatchery-origin steelhead spawning outside the hatchery exceeds the HRSG 5% threshold in several collection areas

Conversely, when imposing the same four viability criteria on the hatchery-origin population several observations of interest arise. The hatchery population has continued to drop in number despite being physically isolated by the hatchery from the effects of hybridization and smolt-to-adult survival rates have fallen to extremely low levels that threaten longterm viability of the hatchery population. During the past few decades the hatchery population has expanded its spatial range well beyond the desired confines of the hatchery facility as shown by the hybrid density data presented in this report. It also appears that in some areas of the Skagit the HRSG threshold of 5% hatchery fish spawning in natural spawning areas has been violated.

12.6 Fish Management and Steelhead Recovery Considerations

Any change to the current hatchery-origin steelhead management strategy presently in effect for the Skagit basin could have ramifications at several levels spanning hatchery reform, tribal treaty rights, sport fishing opportunities and steelhead recovery efforts. We have identified a number of the most basic hatchery management alternatives available to co-managers that include no change in the existing segregated program, a smolt release reduction, elimination of smolt releases or adoption of an integrated program. The expected outcome illustrates the possible consequences of each of these four scenarios (Table 45).

Table 45. The consequences of four differing hatchery program scenarios on hatchery reform, tribal treaty rights, sport fishing opportunity, and steelhead recovery benefits based on the expected outcomes.

Scenario	Hatchery Reform (HSRG)	Tribal Treaty Rights	Sport Fishery Opportunities	Steelhead Recovery Benefit
No Change In Current Segregated Smolt Production Program	Genetic & ecological interaction levels and hatchery straying ***Unchanged***	***No Change*** Targeted hatchery winter steelhead tribal fishery continues. Wild steelhead "take" issues ***continue to impact*** harvest of co-occurring non-listed species. Further SAR decline may restrict fishery to ensure egg take goal.	***No Change*** to current sport fishing opportunities unless SAR levels continue to fall. ***Seasonal restrictions*** may be required to ensure annual egg-take goals.	***No recovery benefit***. Genetic & ecological interaction levels and hatchery straying continue at current levels.
Reduction In Smolt Production	Genetic & ecological interaction levels and hatchery straying ***Reduced***	***Reduced harvest opportunity –***less hatchery steelhead for harvest. Wild steelhead "take" issues ***continue to impact*** harvest of co-occurring non-listed species unless wild steelhead population recovers to de-listing levels	***Less harvestable fish***. If SAR level continues to drop. ***Seasonal restrictions*** may be required to ensure annual egg-take goals.	***Partial recovery benefit***. Genetic & ecological interaction levels and hatchery straying would be ***reduced from present levels.*** Benefits relative to extent of release reduction.

Scenario	Hatchery Reform (HSRG)	Tribal Treaty Rights	Sport Fishery Opportunities	Steelhead Recovery Benefit
Elimination of Smolt Production	Genetic & ecological interaction levels and hatchery straying ***Eliminated***	***Eliminates*** targeted hatchery harvest opportunity. ***Reduces or eliminates likelihood*** of wild steelhead "take" issues impacting harvest of co-occurring non-listed species if wild steelhead population recovers to de- listing levels. A recovered wild population could create a future fishery opportunity.	***Eliminates*** current sport fishing opportunity. A recovered wild population could create a future sport fishery opportunity.	***Full recovery benefit***. Genetic & ecological interaction levels and hatchery straying would be ***eliminated***. Greatest recovery benefits.
Adopt Integrated Program	**New Issues** – New set of possible genetic, ecological and harvest management issues and considerations.	***Eliminates*** targeted hatchery-origin harvest opportunity. ***Uncertainty how this might reduce or eliminate* likelihood of** wild steelhead "take" issues **impacting** harvest of co-occurring non-listed species. Uncertain wild recovery benefits.	***Eliminates*** current sport fishing opportunity. **May create** a future fishery (see Chilliwack example)	***Uncertain recovery benefit***. ***Any recovery benefits would be dependent on program size and hatchery rearing and release protocols***

12.7 Recommended Actions and Validation Steps

There are many factors that collectively contribute to Puget Sound steelhead population declines by altering survival and productivity. At a coarse scale these factors span a variety of physical, biological, regulatory and man-caused factors such as unfavorable ocean conditions and harmful hatchery practices. Recognizing that many factors likely contribute to the decline of Skagit steelhead, this study focused on a single, yet important, factor described as the potential effects of hatchery releases on natural origin steelhead survival and abundance from an ecological and genetic standpoint. Our conclusion and recommendations described below are founded on the science-based body of evidence presented in this report's meta-analysis.

The study results indicate that the segregated hatchery steelhead program currently operating in the Skagit may be negatively impacting the Skagit wild steelhead population. This conclusion is based on potential competition for food and space among hatchery and wild juveniles, and evidence demonstrating gene flow between hatchery and wild populations. In light of this conclusion, we make four recommendations that should be considered within the context of developing a Skagit Steelhead Recovery Plan chapter for the Puget Sound Steelhead DPS. The recommendations are:

1. Discontinue the segregated hatchery steelhead program in the Skagit and monitor the results for 7-10 years. Expected responses of this recommendation are three-fold: a) elimination of hatchery and wild steelhead competition at all life stages b) a rapid reduction of hybridization rates between hatchery and wild steelhead, and c) an increase in survival of wild steelhead.
2. Determine the effects (benefits / risks) of implementing an integrated hatchery steelhead program on wild steelhead recovery and incidental steelhead catch during directed salmon harvest (e.g., spring Chinook, sockeye). The segregated hatchery method is adverse for wild steelhead recovery but an integrated approach to hatchery steelhead production may be beneficial or neutral to wild steelhead recovery.
3. Develop a life stage specific model (Species Life Cycle Analysis Modules - aka SLAM) for Skagit steelhead to predict the consequences of integrated recovery plan actions from all H's (i.e., Hatcheries, Harvest, Habitat, and Hydropower).
4. Determine the benefits of basin wide habitat restoration/protection on the wild steelhead population in the Skagit. New juvenile steelhead density results stratified by freshwater habitat type and season from the Skagit Yearling Study would be used to populate a Skagit steelhead SLAM model.

Recommendation 1 (i.e., discontinue the segregated hatchery steelhead program in the Skagit basin) incorporates several specific effectiveness monitoring actions that are necessary to validate expected outcomes. These effectiveness monitoring actions are:

Action 1 – Discontinue the release of hatchery steelhead smolts from the Skagit Marblemount Hatchery.

Action 2 – Re-analyze dna from this study using SNP's to eliminate assignment error for F1 hybrid individuals as described by Warheit in section 10 of this report. This analysis would be used to quantify introgressive hybridization with a much higher level of confidence than is currently possible with analytic tools current available.

Action 3 – Assemble Skagit adult steelhead abundance data on an annual basis to monitor change over time on both the Skagit and in neighboring basins such as the Keogh River.

Action 4 – On an annual basis, collect dna from Skagit juvenile natural origin steelhead from tributary and mainstem smolt traps to measure any incremental change in genetic introgression over time at the juvenile lifestage. Collect dna samples from Skagit adult natural origin steelhead on an annual basis from within the study reaches used during this study to again measure incremental changes in genetic introgression at the adult lifestage. Other researchers found that hybrid proportions in both smolts and adults shifted quickly relative to the number of naturally spawning hatchery fish present within the watershed. Thus, we expect data from Action 4 would be used to measure the observed "rate of change" in reduced hybrid proportions rather than only taking one measurement after 7 years as described in Action 5.

Action 5 – After a period of 7-10 years repeat the dna sampling by area and lifestage used in the this study to comprehensively measure basin-wide effects of the absence of hatchery influence on genetic introgression levels and hybrid densities on a spatial level within the Skagit basin.

Action 6 – At the conclusion of the validation period fish managers should assemble results derived from Actions 3-5 to test wild Skagit steelhead response to elimination of the segregated steelhead hatchery program in the Skagit. Results should focus on these three predicted responses: a) reduction in genetic introgression levels between hatchery and wild steelhead at the juvenile and adult lifestages, b) absence of naturally spawned hatchery origin steelhead at both the juvenile or adult lifestage (using SNPs), and c) improved adult Skagit wild steelhead abundance relative to other nearby basins and in particular the Keogh River.

We make Recommendations 2 through 4 in light of Recommendation 1 knowing that fishery managers need options to achieve fishery objectives and tools to plan for wild steelhead recovery in Puget Sound under ESA. Thus, we make recommendations 2 through 4 in order to integrate all potential factors of decline of Skagit steelhead (i.e., recommendations 3 and 4) and explore whether different steelhead hatchery approaches may be useful in the Skagit (recommendation 2).

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14.0 Appendices

14. 1 Historical Review of Skagit Steelhead Escapement Goals

A. Historical Review of Skagit Steelhead Escapement Goals

For all intents and purposes, steelhead management on the Skagit started in 1978. Prior to that time there were indeed opening dates and closing dates and bag limits, but sport catch estimates were highly questionable, tribal catches before 1974 were clandestine, and there were no estimates of run size or spawning escapement, and therefore no efforts to manage catches to achieve an escapement goal. Programs to estimate spawning escapement and run size, and a creel census to estimate sport catch, were implemented for the first time during the 1977-78 season.

At that time, the only data available with which to calculate harvestable numbers were previous catches and hatchery smolt releases; consequently, harvestable numbers were set equal to what had been caught in previous seasons, or as the hatchery smolt release multiplied by an average catch per smolt release. Harvestable numbers were not calculated for wild steelhead, and fisheries were front-loaded to avoid wild steelhead. It was assumed that the catches of wild steelhead during the early-season fisheries would be compensated for by an equal number of hatchery steelhead that would spawn in the wild. After 3 years of estimating wild escapements, in 1980 the Washington Department of Game (WDG) proposed a wild escapement goal of 7,000 for the Skagit (Table below), which could be made up of both wild and hatchery fish. The next year, they proposed to increase that goal to "8,000 to 14,000", and in 1982 the goal was simply 8,000.

In 1983, following personnel changes, WDG conducted an analysis of stream area, and announced that, based on these measurements, steelhead were grossly underescaped throughout Puget Sound[1], and that there should be no steelhead fishing anywhere. This brought an outcry not only from the tribes, but also from the public, who demanded to know why they were spending money producing hatchery steelhead, when they wouldn't be allowed to catch them. WDG relented, and proposed setting the Skagit escapement goal at 9600, which was the highest previously-observed escapement, and which appeared subjectively to achieve adequate penetration throughout the system, and allowing a 10% harvest rate if run size was less than the goal. The tribes, who had been stung by salmon escapement goals that were set using inadequate data and then became immutable, resisted establishing a numerical goal for steelhead until more data were acquired, and proposed instead that fisheries continue to be front-loaded to avoid wilds, with a harvest rate ceiling of 45%, until an MSY escapement level could be calculated. Faced with this disagreement, a monumental Case Area-wide Fisheries Advisory Board meeting was held on December [], which included a surprise appearance by Santa Claus, and for which no recommendation was ever issued. In the fallout from this meeting, the state and tribes agreed not to define an escapement goal that year, and set the harvestable number equal to the hatchery run size. The state then unilaterally desequestered the adipose fin for steelhead, and started ad-clipping all hatchery steelhead releases. Since the ad-clipped hatchery fish wouldn't recruit to fisheries until the 1985-86 season, the sport regulations in 1983-84 and 1984-85 required anglers to release steelhead whose dorsal fin height was greater than approximately the width of a credit card.

In order to address the escapement goal dispute, the state and tribes formed a joint technical group to study the issue. There were a handful of rivers with spawning escapement and recruitment data, but few of them had more than two or three spawner-recruit data points, which were too few data points for a river-specific spawner-recruit analysis. The technical group therefore tried to increase

[1] The Skagit wild escapement goal that resulted from this exercise was 31,000.

the number of data points by constructing a Case Area-wide spawner-recruit relation that included all the points from all the rivers in one graph . This required the group to standardize spawner-recruit data across all the rivers (e.g., calculate how many Quillayute Rivers equals one Skagit River, and expand the Quillayute data by that factor). This exercise was completed in early 1985 -- the results of this calculation, however, were highly sensitive to some untestable assumptions. While the state was willing to accept these uncertainties[2], the tribes were not, and an impasse loomed again. The impasse was eventually resolved by a policy agreement that the harvestable number would be 91.8% of the hatchery run, plus 2500 wild steelhead. This agreement was renewed annually through 1991. Thus, the wild escapement goal from 1986-1991 was to manage for a wild harvest of 2,500 (i.e., the escapement would be Wild Runsize minus 2,500). The 2,500 harvest was a negotiated number that the co-managers agreed was low enough to allow the high escapements that would test system capacity and productivity. This strategy was successful, as 4 of the following 5 escapements exceeded 10,000.

A prolonged drought, however, occurred in the late 1980s, and the 1991 wild escapement, and the 1991-92 wild preseason forecast, were both considerably lower than previous levels. This presented WDG with a particular dilemma because, although the 10,300 escapement goal they had proposed in 1985 was never agreed to by the tribes, or used in management, WDG had nonetheless publicized that number to their constituents as the escapement goal, and their constituents had accepted that as a fact. In 1991-92, however, with a preseason forecast less than 10,300, WDG could no longer justify to their constituents the wild harvestable number of 2,500 without facing a credibility crisis. At the same time, however, commercial buyers began to lose interest in steelhead, and tribal fishing effort also dropped substantially. Because of this decline in tribal effort, the co-managers agreed that haggling over the escapement goal was unnecessary, and agreed instead to fish according to fishing schedules that were set preseason, without defining an escapement goal. This strategy was used during the 1991-92, 1992-93, and 1993-94 fishing seasons (there was also a 2500 fish cap on total tribal catch in 1991-92).

By 1992, there was a sufficient spread of Skagit steelhead escapements, with their resulting recruitments, to perform a spawner-recruit analysis that used <u>only Skagit-specific data</u> (see Comments column for 1992-93 season in table below). Accordingly, the <u>same</u> statisticians who did the 1985 analysis, using the <u>same</u> analytical methods that were used in the 1985 analysis, but (because they used only Skagit-specific data) <u>without</u> using any cross-basin equivalency assumptions (hence, their 1992 analysis was <u>more valid</u> for the Skagit than the 1985 analysis), calculated that the MSY escapement level for Skagit wild steelhead was in the 3,000 to 4,500 range. Because of unease over using these numbers as the goal, the co-managers agreed to continue testing system productivity and capacity, but, in order to avoid increasing harvest impacts if run size dropped, they <u>changed</u> the goal to a harvest rate ceiling. This ceiling, 16%, was the mean harvest rate observed in the previous 6 seasons. By establishing the previous <u>mean</u> rate as the <u>ceiling</u> rate, the effect was to <u>reduce</u> mean harvest rates from what they had been. The purpose of these goals (the ceiling HR of 16%, and, before that, the wild harvest of 2,500) was to keep fishing impacts low enough to test the productivity and capacity of the system, so that we could define better the MSY escapement level. The intent when the 16% HR ceiling was established in 1994 was to continue testing system productivity and capacity for another 4 or 5 years, and then revisit the MSY escapement calculations. If system capacity was greater than the 1992 analysis indicated, then

[2] They proposed a goal of 10,300, which was an average of three different estimates of MSY escapement.

abundance would increase over that time -- if the 1992 analysis was accurate, then abundance would stabilize. The latter is closer to what occurred, as, except for a 2-year drop in 2000 and 2001, neither of which dipped below the MSY escapement range, the run size since 1991 has stabilized in the 4000 to 8000 range.

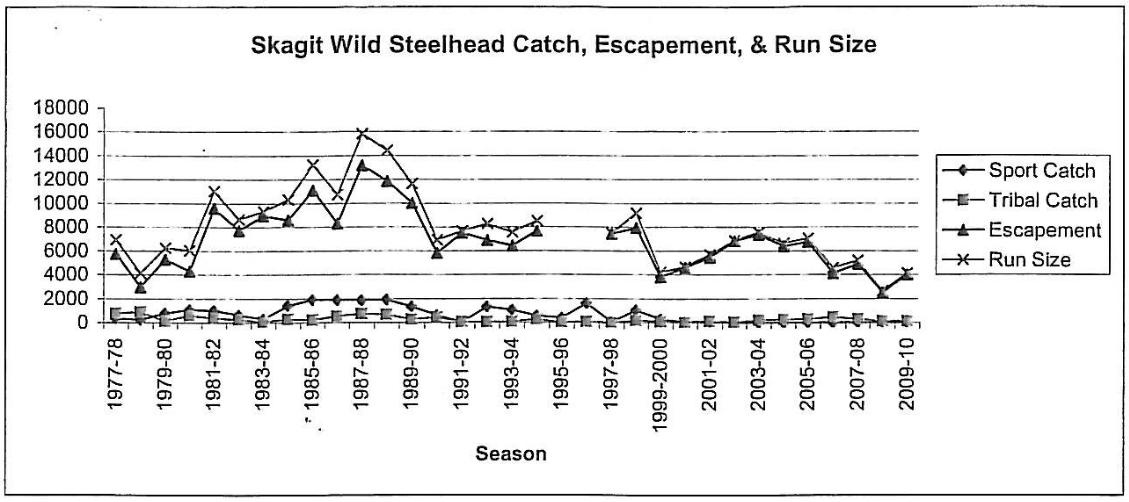
After the run size dropped during the 1999-2000 season, and the 2000-2001 forecast was for a "poor" run size, WDFW regional staff wanted to close the Skagit to wild steelhead retention by sportsmen during the 2000-2001 season. They were told, however, that the Fish and Wildlife Commission would not adopt a closure unless the run size was less than an agreed numerical threshold. Consequently, the co-managers agreed to set 6000, which was comfortably above the MSY escapement range of 3000 to 4500, as the "floor" escapement above which fisheries directed at wild steelhead could be conducted, up to the 16% harvest rate ceiling (the 16% ceiling would continue to apply at all abundance levels). Since the 2000-2001 forecast was below this floor, the Fish and Wildlife Commission closed the Skagit to wild steelhead retention by sportsmen (and did the same thing the next year). Ironically, because this floor is less than 10,300 (which was never a Skagit steelhead escapement goal, and has been refuted by subsequent analyses), some sports groups have criticized the 6000 floor as being set for the purpose of "accommodating fisheries", when, in fact, the purpose of this floor was to close a fishery.

These floor and harvest rate ceiling levels remain the current escapement goal for Skagit wild steelhead.

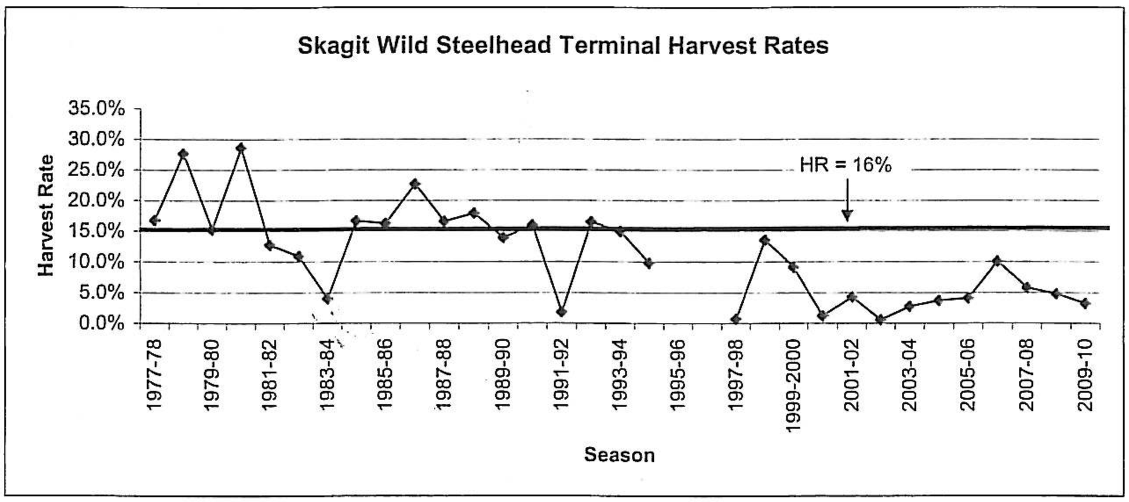
SKAGIT WILD STEELHEAD MANAGEMENT

Season	PSF Wild Run Size	Escapement Goal	Comments
1977-78	No PSF	None	Harvestable = Hatchery run size (HRS)
1978-79	No PSF	None	Harvestable = HRS (10000 in-season update)
1979-80	No PSF	None	Harvestable = HRS (7000). Assume wild catch = hatchery spawners
1980-81	13000 (Total H+W)	7000 (H+W) (WDG)	Total Run Size (RS) = 13000; Harvestable = 13000 – 7000 = 6000.
1981-82	?	8000(-14000) (WDG)	WDG memo referred to "some harvestable wild"
1982-83	7400	8000	Harvestable = 4000 (including 10.8% of wild run as incidentals)
1983-84	WDG = 6500-7000 SSC = 4000-10000	None Agreed	WDG proposed 9600 with 10% harvest rate (HR) if RS<9600. SSC proposed avoiding wilds, with 45% HR ceiling. Agreed plan (Harvestable = HRS and frontload fisheries to avoid wilds) accommodated both parties' goals.
1984-85	10600	None agreed/proposed	Parties awaiting further analyses. Minimize wild catch in meantime. Agreed plan (Harvestable = HRS or close Jan 16 or 34 "equivalent days") accommodated both parties' goals. River test fishery.
1985-86	14500-16000	Wild harvest = 2500	Spawner-recruit report done. WDG proposed 10300 in April 1985. SSC proposed not specifying a number yet. Mass-marks appear.
1986-87	12700-15100	Wild harvest = 2500	
1987-88	11946-15460	Wild harvest = 2500	
1988-89	12500-16000	Wild harvest = 2500	
1989-90	12900-16400	Wild harvest = 2500	Sauk-Suiattle test fishery in Sauk, Jan 2-3, 1990
1990-91	No PSF	Wild harvest = 2500	
1991-92	9303	None Agreed	End of creel census; no Swinomish buyers; no update. Agreed to fish a schedule (18 days Swinomish; 9 days Upper Skagit/Sauk-Suiattle; closed by Jan 31), with 2500 total (H+W) catch ceiling.
1992-93	12300	None Agreed	Skagit spawner-recruit analysis done; it estimated MSY escapement considerably less than in 1985 report. Agreed to schedule (19 days for Swinomish, ending in March; 10 for Upper Skagit/Sauk-Suiattle, ending mid-February), and that there are harvestable wild steelhead.
1993-94	No PSF	None Agreed	Same as 1992-93.
1994-95	8000-10300	<16% harvest rate	Agreed to "unquantified" number of harvestable wild steelhead. Same number of fishing days, with wild non-retention after Feb 28.

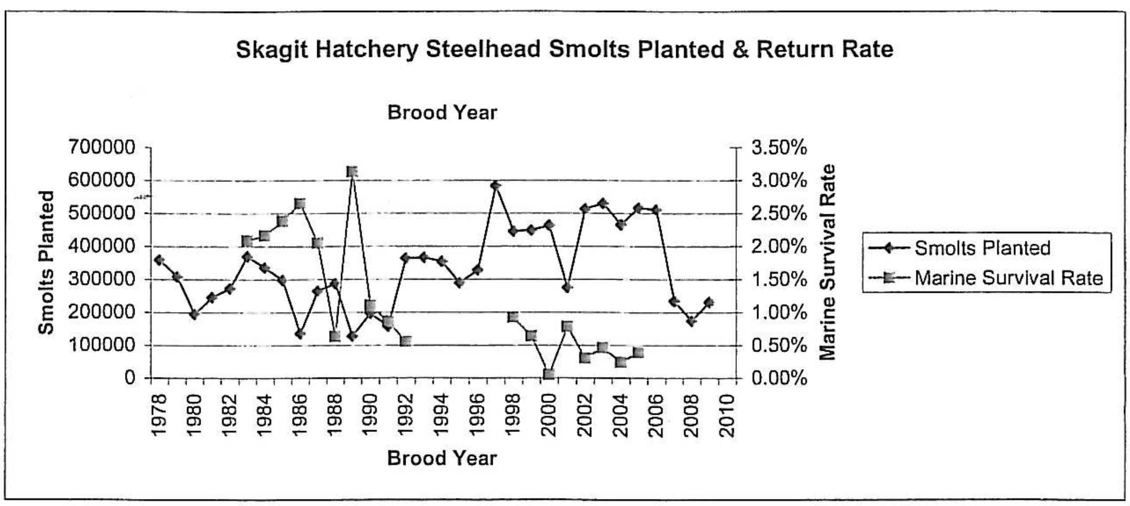
Season	PSF Wild Run Size	Escapement Goal	Comments
1995-96	8800-10800	<16% harvest rate	Same as 1994-95.
1996-97	No PSF	<16% harvest rate	Same as 1994-95.
1997-98	No PSF	<16% harvest rate	Upper Skagit/Sauk-Suiattle increased to 12 days; dropped Feb 28 closure. WA Trout analysis of 1992 spawner-recruit report arrives at similar estimate of MSY escapement.
1998-99	"same as recent" (7000-9000)	<16% harvest rate	Upper Skagit/Sauk-Suiattle increased to 14 days; open to mid-March.
1999-2000	"same as recent" (7000-9000)	<16% harvest rate	Same number of fishing days; Upper Skagit/Sauk-Suiattle open to end of March. Needed special notice for enforcement.
2000-01	"poor" egs: 2400 & 4200	<16% harvest rate with 6000 floor	Same number of fishing days; tribes closed mid-February; sports wild release to end of February, then complete closure (except forks).
2001-02	3000-6000	<16% harvest rate with 6000 floor	Same number of fishing days; tribes closed by end of February; sports same as 2000-01.
2002-03	3500-7700	<16% harvest rate with 6000 floor	Same schedule for Swinomish; Upper Skagit/Sauk-Suiattle open 13 days at Tribes' discretion
2003-04	6162	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 19 days, ending Feb 7; Upper Skagit targeted catch of 300-343 @ ≤ 2.5 days/wk until end of sport fishery
2004-05	6854	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 33 days, ending mid-March; Upper Skagit schedule 36 days to end of March, or catch of 553 steelhead, whichever occurs first.
2005-06	6622	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 34.67 days, ending mid-March; Upper Skagit open 32.17 days, ending April 15.
2006-07	7054	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 32.5 days, ending mid-March; Upper Skagit open 33.08 days, ending April 30.
2007-08	5061	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 30.4 days, ending mid-March; Upper Skagit open 31.33 days, ending April 30.
2008-09	7499	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 32.5 days, ending mid-March; Upper Skagit open 25.67 days, ending April 15.
2009-10	5739	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle open 30.7 days, ending mid-March; Upper Skagit open 36.5 days, ending April 15.
2010-11	4201	<16% harvest rate with 6000 floor	Swinomish/Sauk-Suiattle and Upper Skagit open 21.67 days, ending early February.



Skagit Wild Steelhead Catch, Escapement, & Run Size
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Sport Catch
Tribal Catch
Escapement
Run Size
1977-78
1979-80
1981-82
1983-84
1985-86
1987-88
1989-90
1991-92
1993-94
1995-96
1997-98
1999-2000
2001-02
2003-04
2005-06
2007-08
2009-10
Season



Skagit Wild Steelhead Terminal Harvest Rates
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Harvest Rate
HR = 16%
1977-78
1979-80
1981-82
1983-84
1985-86
1987-88
1989-90
1991-92
1993-94
1995-96
1997-98
1999-2000
2001-02
2003-04
2005-06
2007-08
2009-10
Season



Skagit Hatchery Steelhead Smolts Planted & Return Rate
Brood Year
700000
600000
500000
400000
300000
200000
100000
0
Smolts Planted
3.50%
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
Marine Survival Rate
Smolts Planted
Marine Survival Rate
1978
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
2010
Brood Year

SKAGIT STEELHEAD RUN SIZE DATA

	WILD RUN					HATCHERY RUN					
Season	Sports Catch	Tribal Catch	Spawning Escapemt	Run Size	Harvest Rate	Sports Catch	Tribal Catch	Spawning Escapemt	Run Size	Smolts (BY)	Return Rate
1977-78	371	787	5757	6915	16.7%	3033	3465	1537	8035	358955	
1978-79	240	901	2982	4123	27.7%	4638	3986	961	9585	308321	
1979-80	799	154	5288	6241	15.3%	2679	4046	721	7446	194697	
1980-81	1105	623	4308	6036	28.6%	1231	2364	1127	4722	245393	
1981-82	1023	384	9609	11016	12.8%	1635	2313	735	4683	271793	
1982-83	666	281	7732	8679	10.9%	632	1700	434	2766	370017	2.09%
1983-84	296	79	8963	9338	4.0%	1698	3228	917	5843	336417	2.16%
1984-85	1435	283	8603	10321	16.6%	4793	4690	3702	13185	298357	2.38%
1985-86	1916	233	11098	13247	16.2%	2525	4665	1339	8529	136096	2.65%
1986-87	1895	536	8305	10736	22.6%	1646	3530	964	6140	264376	2.05%
1987-88	1873	746	13194	15813	16.6%	2255	4161	1195	7611	286833	0.63%
1988-89	1905	676	11854	14435	17.9%	1217	2964	779	4960	127032	3.13%
1989-90	1351	272	10017	11640	13.9%	1283	3227	840	5350	196893	1.10%
1990-91	637	465	5818	6920	15.9%	141	1681	339	2161	157842	0.86%
1991-92	53	84	7514	7651	1.8%	974	2309	611	3894	364161	0.55%
1992-93	1318	46	6900	8264	16.5%	1721	749	460	2930	366591	
1993-94	1056	74	6412	7542	15.0%	596	173	143	912	354122	
1994-95	561	271	7656	8488	9.8%	981	944	355	2280	289052	
1995-96	402	22				913	527	49	1489	328461	
1996-97	1622	73				1847	160	67	2074	583720	
1997-98	49	3	7448	7500	0.7%	347	139	464	950	445434	0.92%
1998-99	1055	191	7944	9190	13.6%	576	105	449	1130	449302	0.63%
1999-2000	284	99	3810	4193	9.1%	437	152	666	1255	463460	0.05%
2000-01	53	4	4591	4648	1.2%	1569	108	154	1831	273712	0.78%
2001-02	132	111	5431	5674	4.3%	3010	188	376	3574	513330	0.30%
2002-03	0	40	6818	6858	0.6%	623	39	710	1372	529821	0.46%
2003-04	0	209	7332	7541	2.8%	1026	126	151	1303	466100	0.24%
2004-05	1	246	6382	6630	3.7%	803	484	523	1810	517000	0.39%
2005-06	2	287	6757	7046	4.1%	876	98	440	1414	511560	
2006-07	4	457	4113	4574	10.1%	1275	896	506	2677	235010	
2007-08	2	300	4887	5189	5.8%	1442	350	423	2215	174000	
2008-09	2	125	2502	2629	4.8%	413	227	246	886	231500	
2009-10	10	124	4003	4136	3.2%	527	317	155	999		

14. 2 Hatchery Smolt Predation Data

Table 14.2.1. 2009 SK Hatchery Steelhead Smolt Predation Data.

Sample Number	Date	Length (mm)	Steelhead	Chinook	Chum	Unknown Salmonid	Other Fish	Coho	Pink	Lamprey	Leech	Damsel Fly	Mayfly	Caddis	Stonefly	Beetle	Total Items
1	5/14/2009	174															0
2	5/14/2009	183											1				1
3	5/14/2009	171										1					1
4	5/15/2009	190															0
5	5/15/2009	196															0
6	5/15/2009	186															0
7	5/15/2009	189															0
8	5/15/2009	183															0
9	5/15/2009	166													1		1
10	5/15/2009	170															0
11	5/15/2009	161			1												1
12	5/15/2009	167			1												1
13	5/16/2009	186				1											1
14	5/16/2009	206			2												2
15	5/16/2009	188												1			1
16	5/16/2009	189													2		2
17	5/17/2009	197								1			2				3
18	5/17/2009	196						1									1
19	5/17/2009	200		1	1							1					3
20	5/17/2009	187															0
21	5/17/2009	186															0
22	5/17/2009	184		1	1	1											3
23	5/17/2009	172															0
24	5/17/2009	171										1					1
25	5/17/2009	172															0
26	5/17/2009	166						1									1
27	5/17/2009	158												1			1
28	5/18/2009	217										1					1
29	5/18/2009	196										1					1
30	5/18/2009	186		1		1		1									3
31	5/18/2009	190													1		1
32	5/18/2009	176													1		1
33	5/18/2009	150															0
34	5/22/2009	186													1		1
35	5/22/2009	188		1								1					2
36	5/22/2009	180				2									1		3
37	5/22/2009	177			3	1											4
38	5/22/2009	190											1	1	1		3
39	5/22/2009	168		2	1	2						4					9
40	5/22/2009	172		1	1	1											3

Sample Number	Date	Length (mm)	Steelhead	Chinook	Chum	Unknown Salmonid	Other Fish	Coho	Pink	Lamprey	Leech	Damsel Fly	Mayfly	Caddis	Stonefly	Beetle	Total Items
41	5/23/2009	198		1													1
42	5/23/2009	158															0
43	5/23/2009	183			1	1											2
44	5/23/2009	183		1	2												3
45	5/23/2009	150		1	2												3
46	5/25/2009	190												1	2		3
47	5/25/2009	194											2				2
48	5/25/2009	176		2	1	1											4
49	5/25/2009	187										1			1		2
50	5/25/2009	176										1			1		2
51	5/25/2009	148		1													1
Totals			0	13	17	11	0	3	0	1	0	12	6	4	12	0	79

Table 14.2.2. 2010 SK Hatchery Steelhead Smolt Predation Data

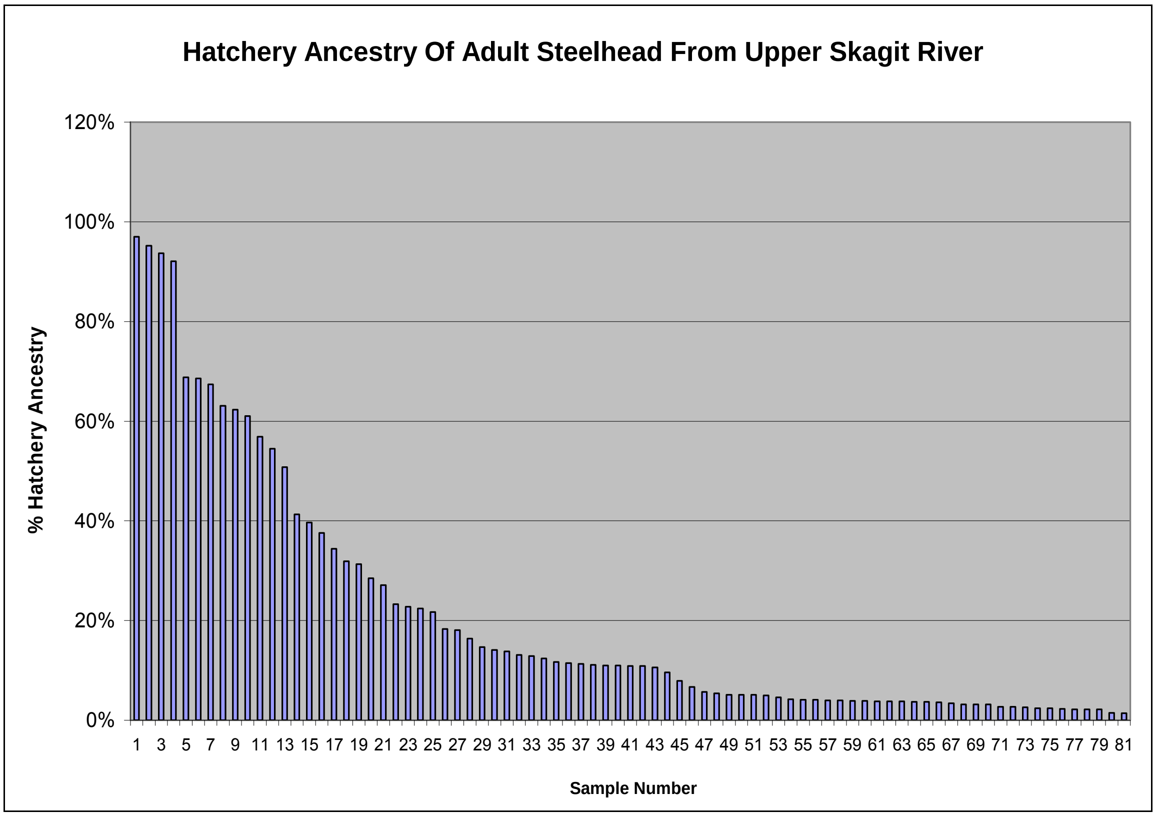
Sample Number	Date	Length (mm)	Steelhead	Chinook	Chum	Unknown Salmonid	Other Fish	Coho	Pink	Lamprey	Leech	Caddis	Stonefly	Beetle	Total Items
1	5/6/2010	180							5						5
2	5/6/2010	161							6						6
3	5/6/2010	190							4						4
4	5/6/2010	155							7						7
5	5/6/2010	143							2						2
6	5/14/2010	197													0
7	5/14/2010	213							10					1	11
8	5/14/2010	217							14						14
9	5/14/2010	206													0
10	5/14/2010	190							8						8
11	5/15/2010	190							16						16
12	5/15/2010	200							11						11
13	5/15/2010	160							8						8
14	5/15/2010	180							8						8
15	5/15/2010	175			1				12						13
16	5/17/2010	163													0
17	5/17/2010	187									1				1
18	5/17/2010	215										1			1
19	5/17/2010	160							1						1
20	5/17/2010	205										1			1
21	5/17/2010	210													0
22	5/17/2010	207										1			1
23	5/17/2010	173										1			1
24	5/17/2010	202										1			1
25	5/21/2010	185													0
26	5/21/2010	205										1			1

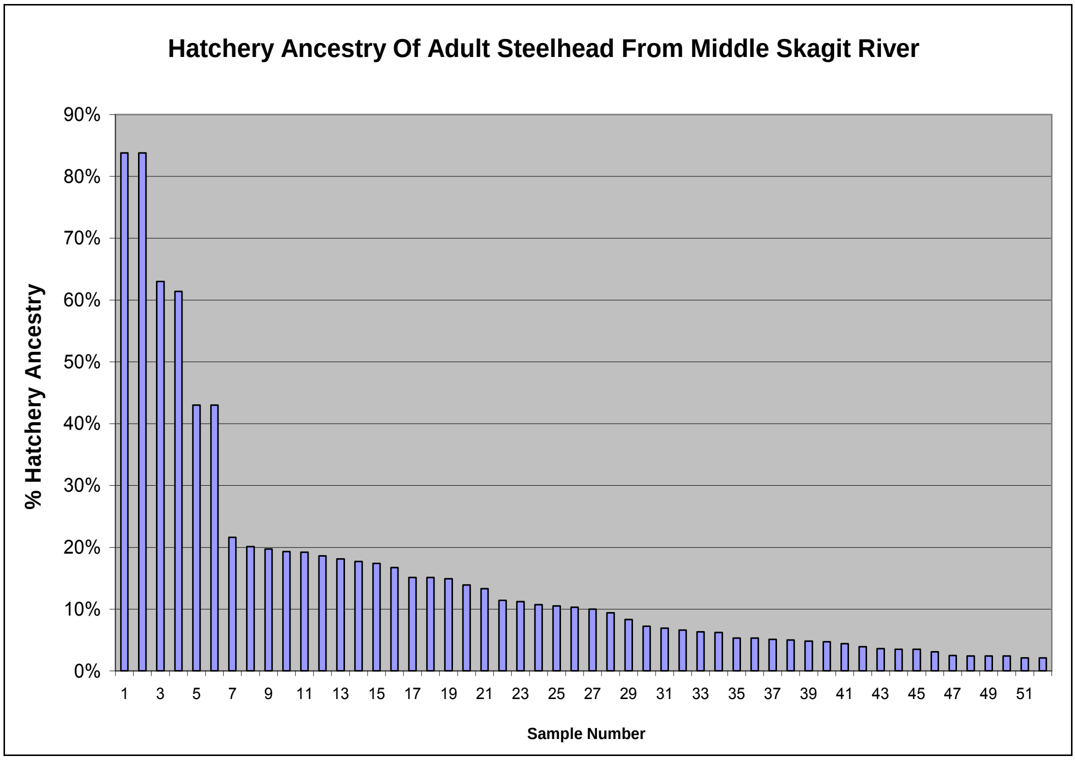
Sample Number	Date	Length (mm)	Steelhead	Chinook	Chum	Unknown Salmonid	Other Fish	Coho	Pink	Lamprey	Leech	Caddis	Stonefly	Beetle	Total Items
27	5/21/2010	180						2							2
28	5/21/2010	163											1		1
29	5/21/2010	190							1						1
30	5/22/2010	193											1		1
31	5/22/2010	157													0
32	5/22/2010	177													0
33	5/22/2010	156													0
34	5/22/2010	173										1			1
35	5/23/2010	176							2			1			3
36	5/23/2010	154													0
37	5/23/2010	180							6						6
38	5/23/2010	155							6						6
39	5/23/2010	187													0
40	5/23/2010	184													0
41	5/24/2010	195										1	1		2
42	5/24/2010	183						1							1
43	5/24/2010	150									1	1			2
44	5/24/2010	203							11						11
45	5/24/2010	179							7						7
46	5/25/2010	163													0
47	5/25/2010	175					1		4	1					6
48	5/25/2010	160							3						3
49	5/25/2010	142													0
50	5/25/2010	157													0
Totals			0	0	1	0	1	3	152	1	2	10	3	1	174

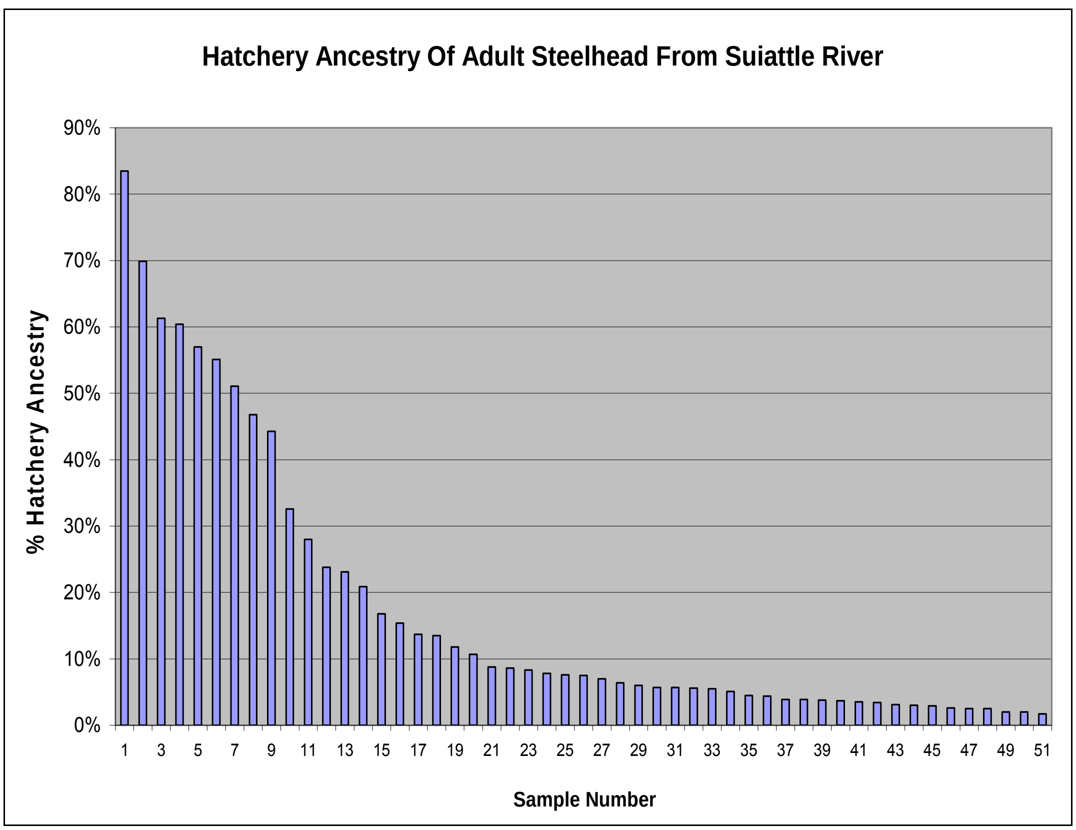
14. 3 Hatchery Ancestry From Individual Adult and Juvenile Steelhead From Various Sampling Locations

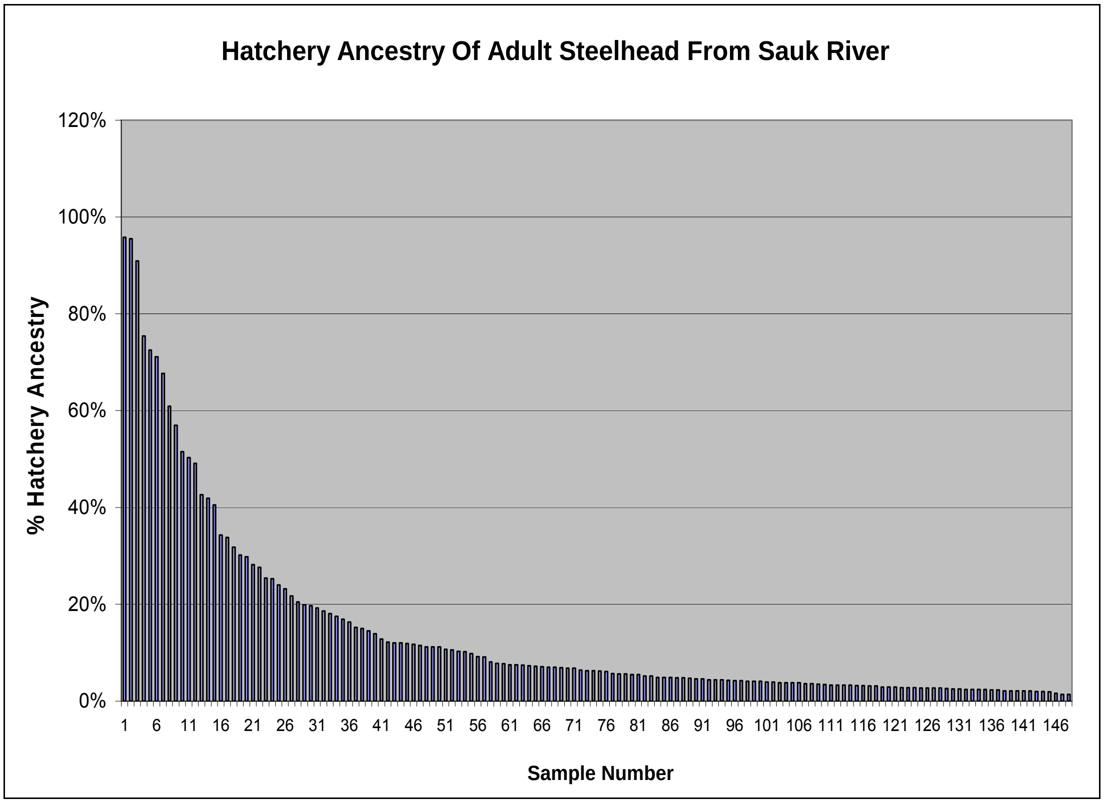
The following graphs are derived from the results of genetic analysis using ***Structure*** to determine the amount of Marblemount hatchery-origin ancestry in individual adult and juvenile steelhead from the sampling regions used in the study report.

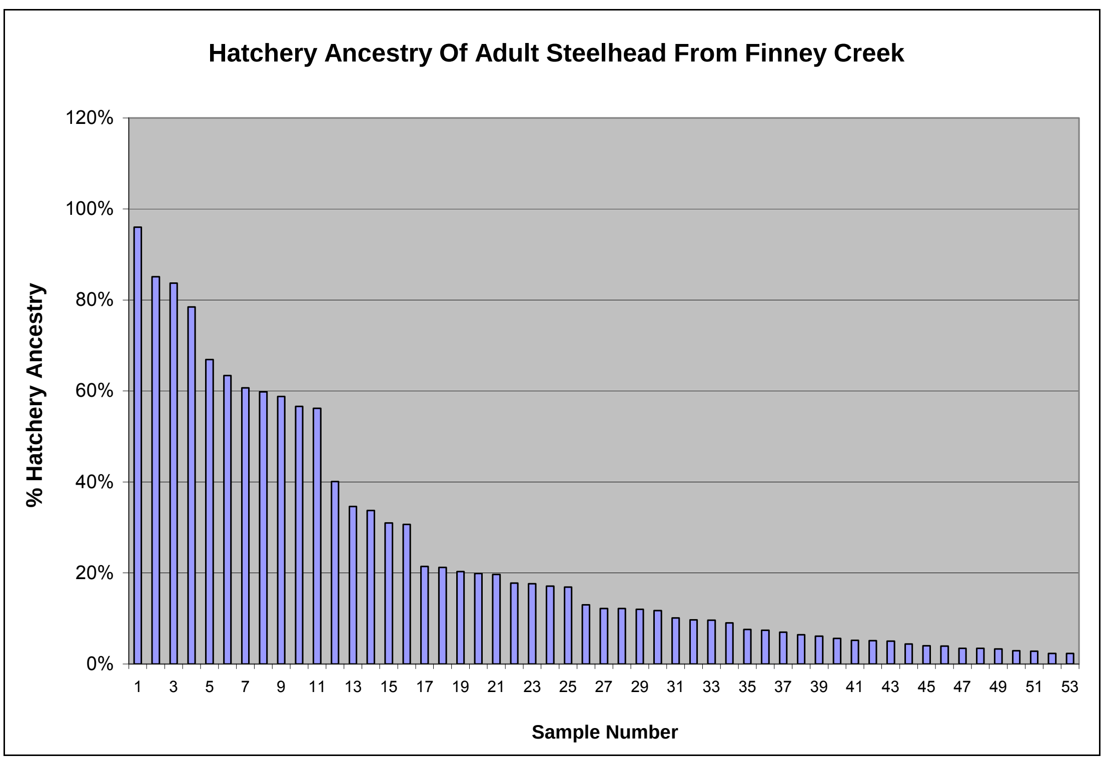
Adult Steelhead

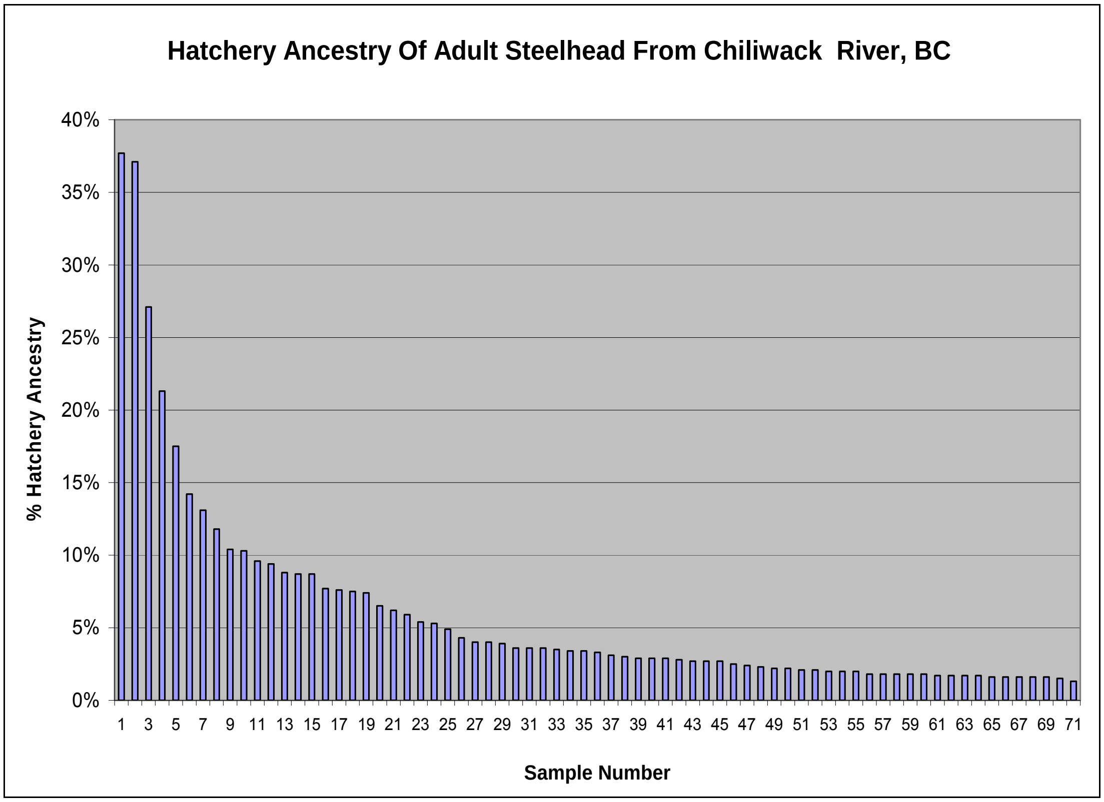


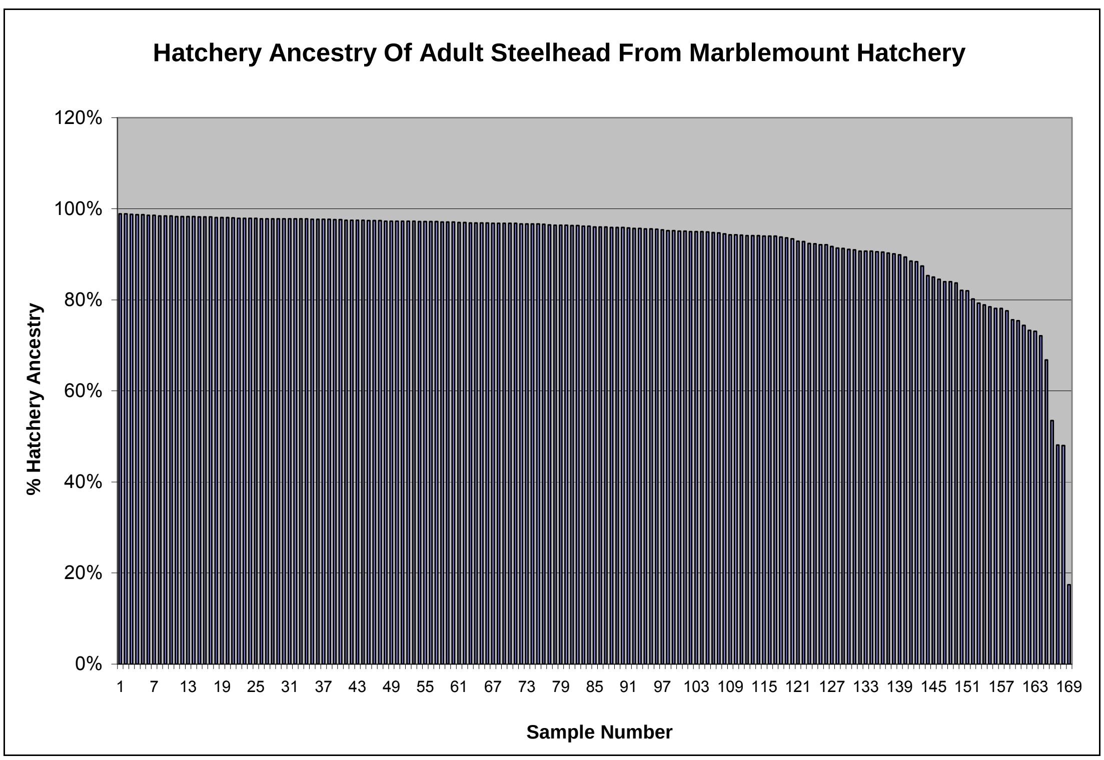

Hatchery Ancestry Of Adult Steelhead From Middle Skagit River
% Hatchery Ancestry
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51
Sample Number


Hatchery Ancestry Of Adult Steelhead From Suiattle River
% Hatchery Ancestry
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51
Sample Number

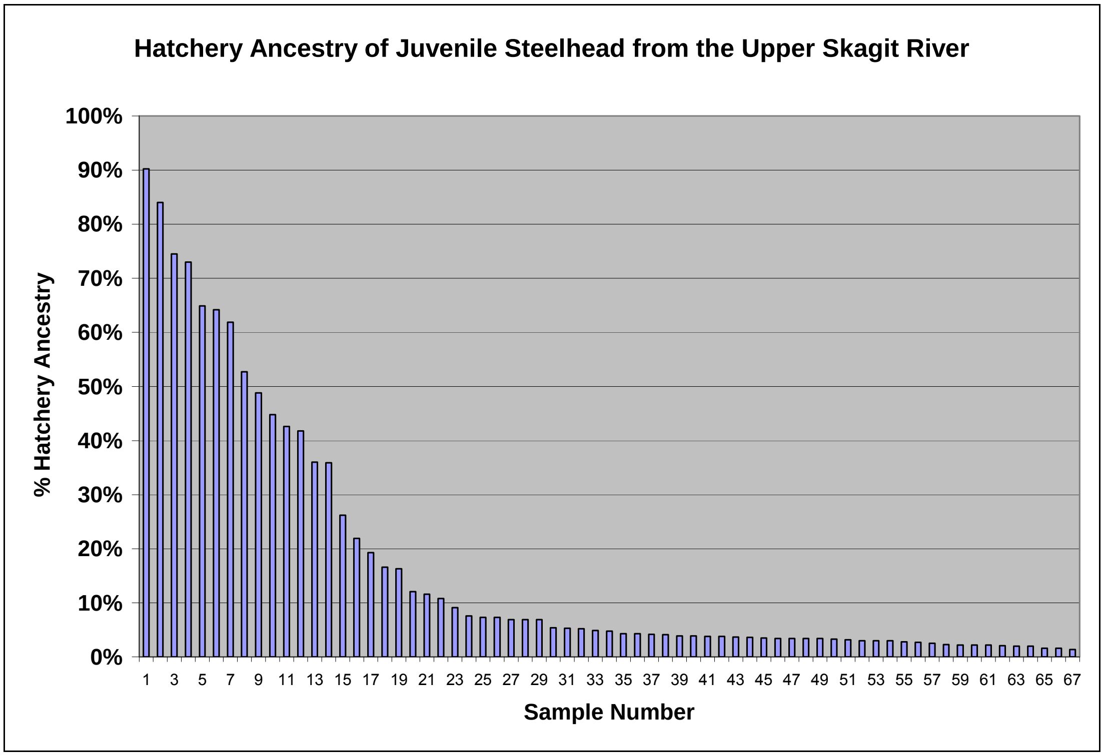

Hatchery Ancestry Of Adult Steelhead From Sauk River
% Hatchery Ancestry
120%
100%
80%
60%
40%
20%
0%
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96 101 106 111 116 121 126 131 136 141 146
Sample Number

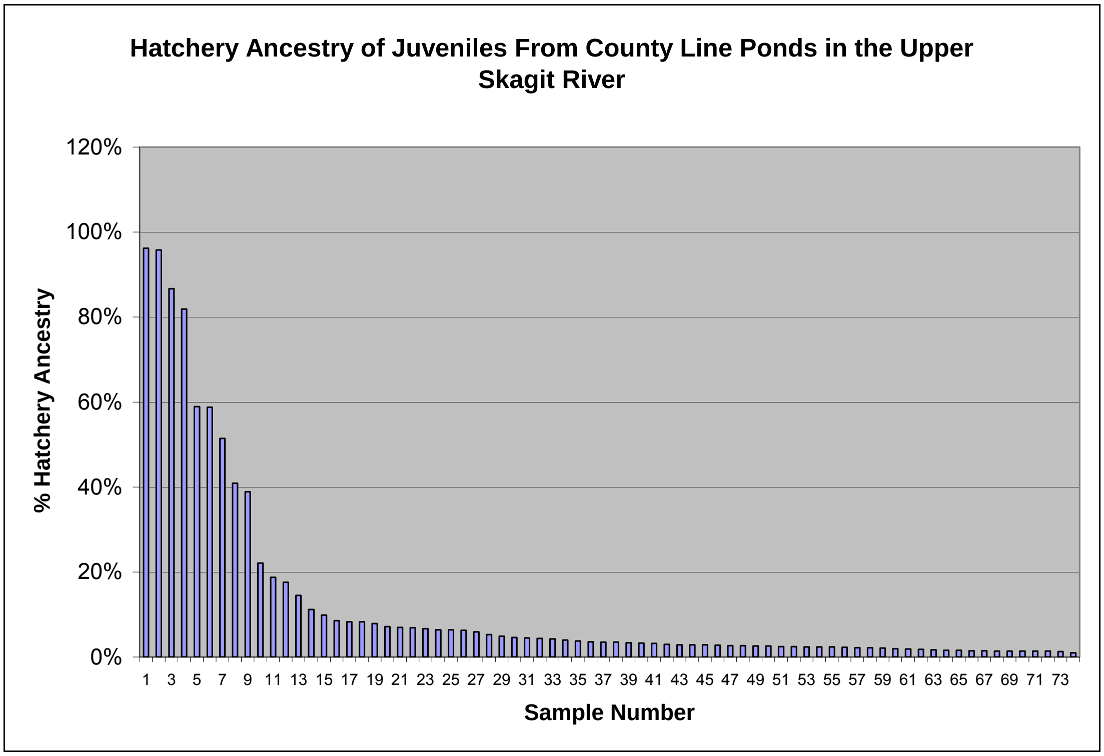

Hatchery Ancestry Of Adult Steelhead From Finney Creek
% Hatchery Ancestry
120%
100%
80%
60%
40%
20%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53
Sample Number

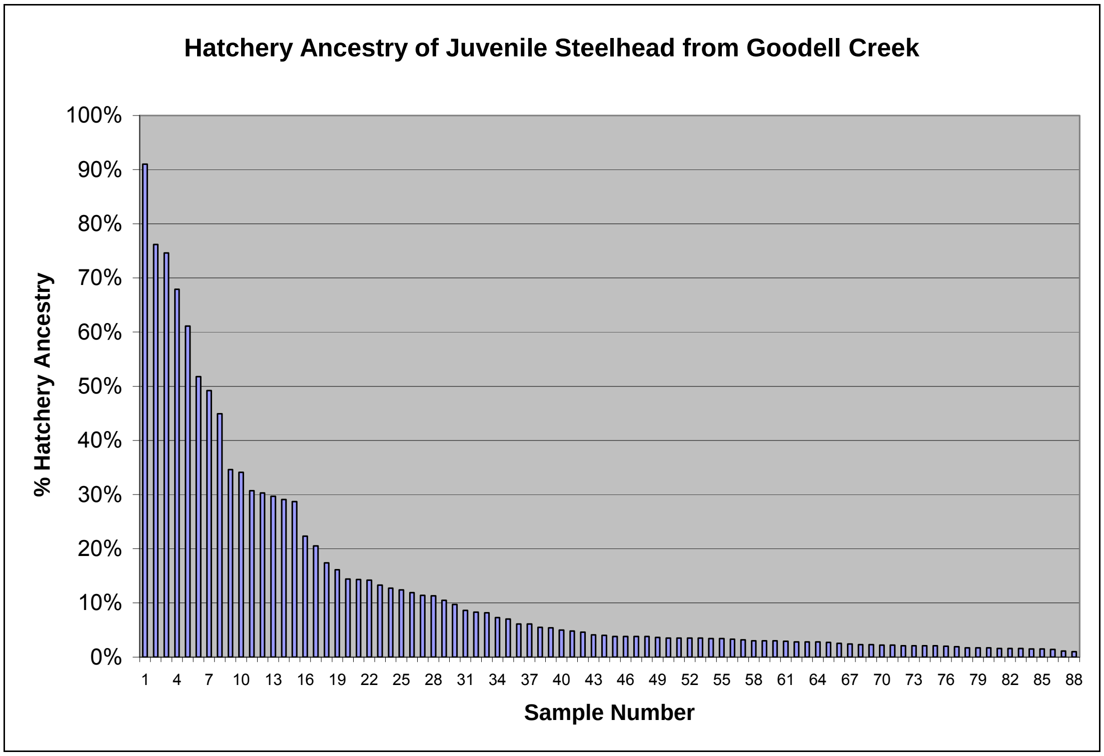

Hatchery Ancestry Of Adult Steelhead From Chiliwack River, BC
40%
35%
30%
25%
20%
15%
10%
5%
0%
% Hatchery Ancestry
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71
Sample Number

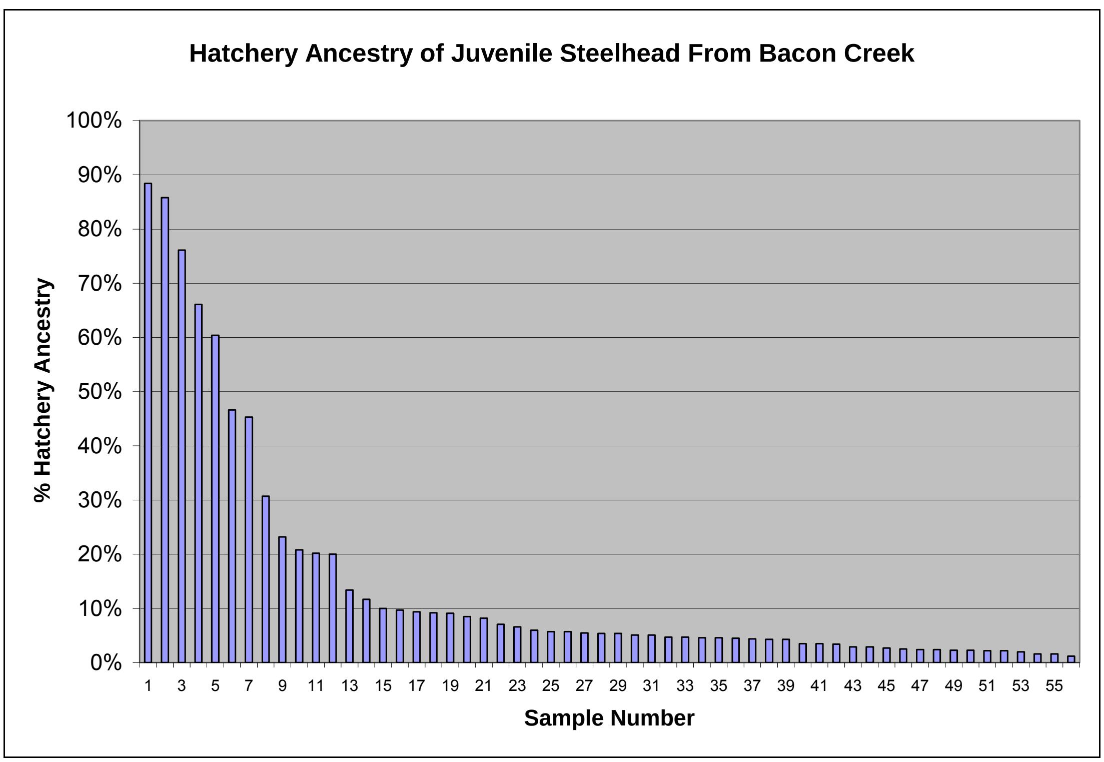

Hatchery Ancestry Of Adult Steelhead From Marblemount Hatchery
120%
100%
80%
60%
40%
20%
0%
% Hatchery Ancestry
1 7 13 19 25 31 37 43 49 55 61 67 73 79 85 91 97 103 109 115 121 127 133 139 145 151 157 163 169
Sample Number

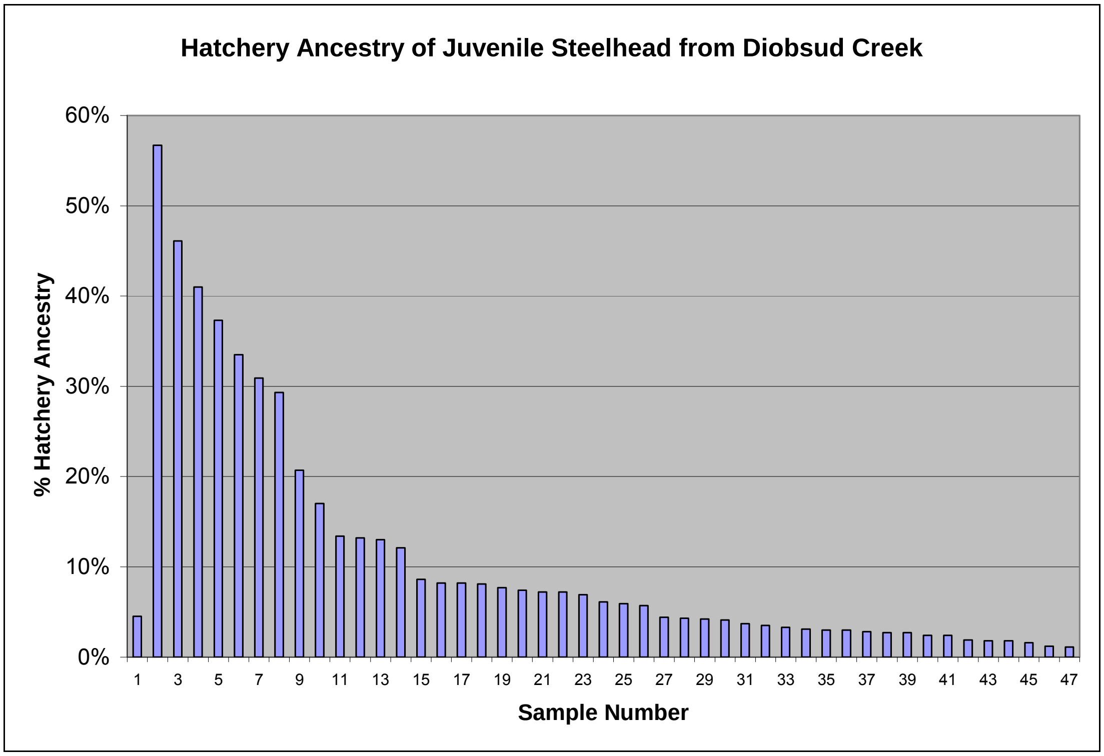
Juvenile Steelhead

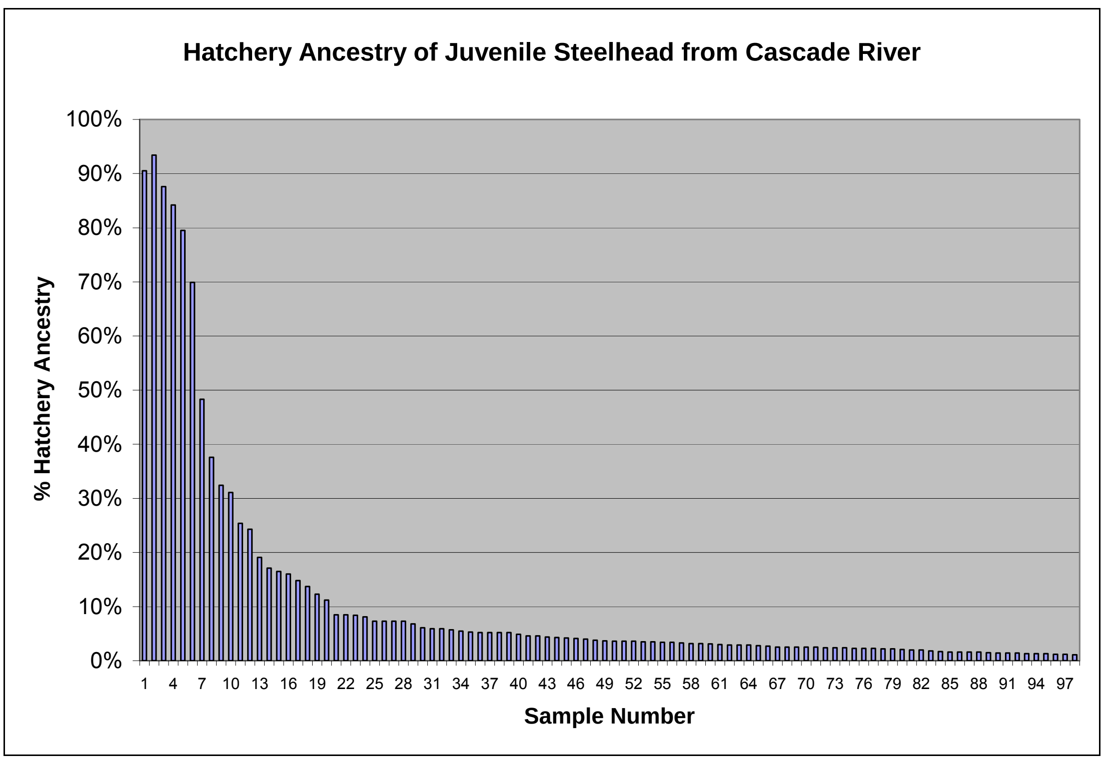


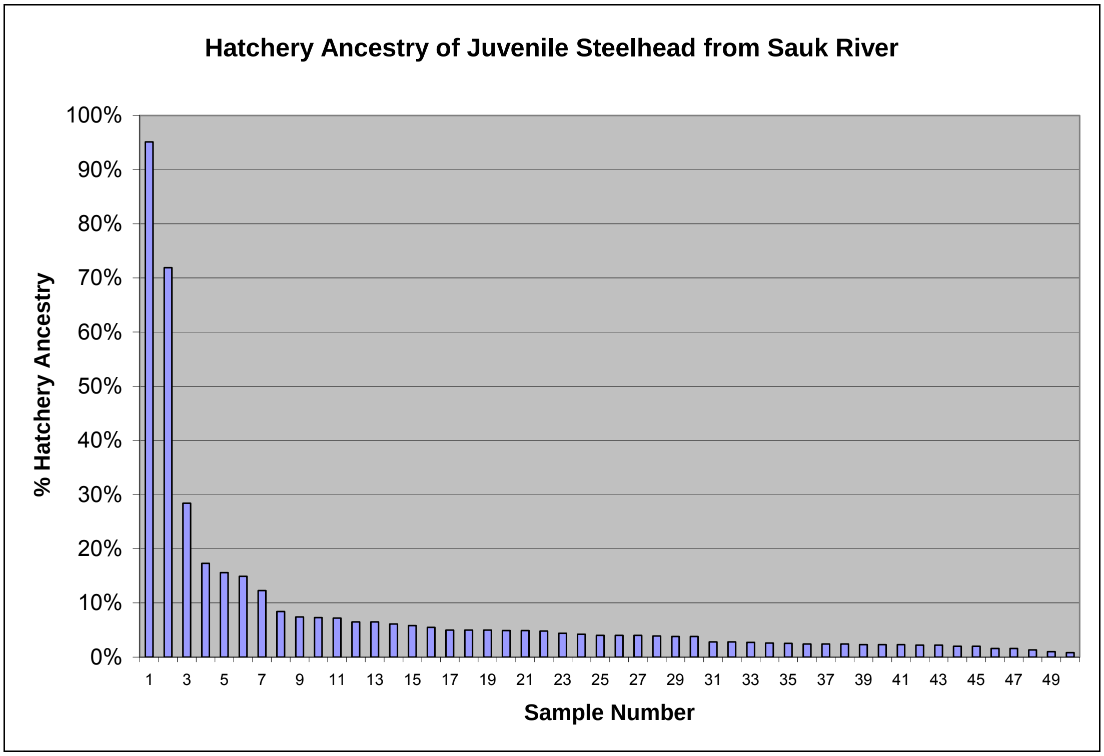


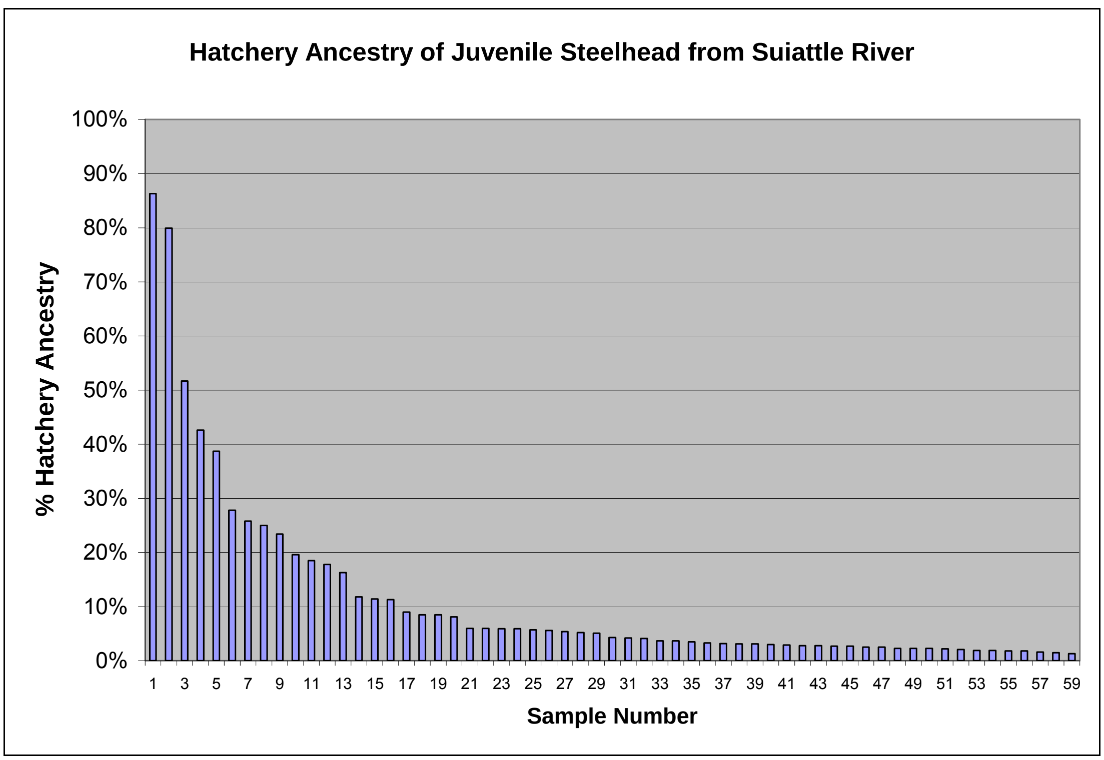

Hatchery Ancestry of Juvenile Steelhead from Goodell Creek
% Hatchery Ancestry
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88
Sample Number

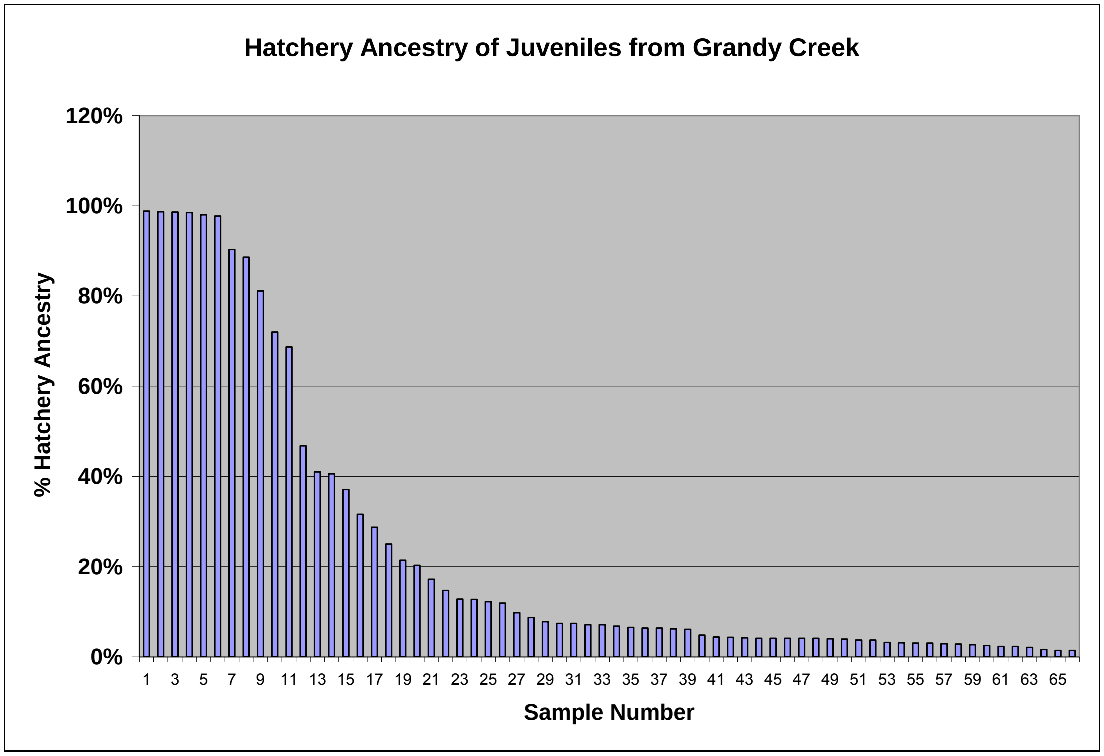

Hatchery Ancestry of Juvenile Steelhead From Bacon Creek
% Hatchery Ancestry
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
Sample Number

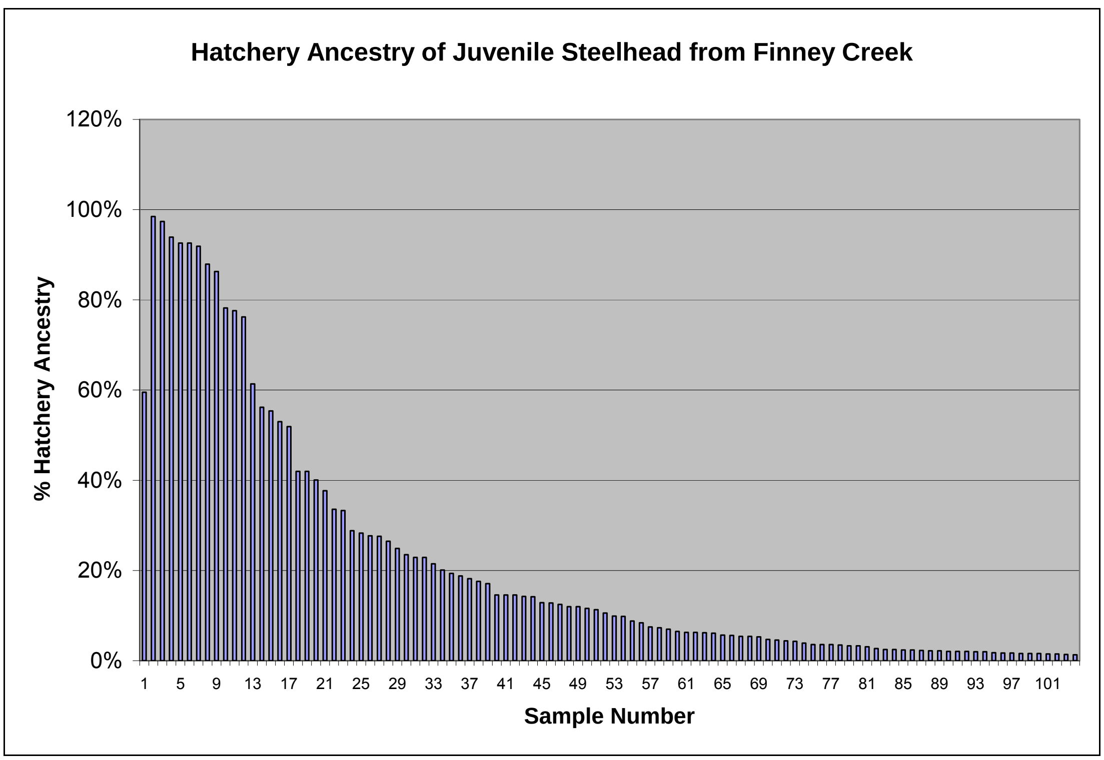

Hatchery Ancestry of Juvenile Steelhead from Diobsud Creek
% Hatchery Ancestry
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
Sample Number


Hatchery Ancestry of Juvenile Steelhead from Cascade River
% Hatchery Ancestry
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97
Sample Number


Hatchery Ancestry of Juvenile Steelhead from Sauk River
% Hatchery Ancestry
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49
Sample Number


Hatchery Ancestry of Juvenile Steelhead from Suiattle River
% Hatchery Ancestry
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Sample Number


Hatchery Ancestry of Juveniles from Grandy Creek
% Hatchery Ancestry
120%
100%
80%
60%
40%
20%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65
Sample Number


Hatchery Ancestry of Juvenile Steelhead from Finney Creek
% Hatchery Ancestry
120%
100%
80%
60%
40%
20%
0%
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101
Sample Number