Progress Report

Genetic-based abundance estimates for Snohomish River Chinook salmon

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Executive Summary

The Snohomish River basin is comprised of two Chinook salmon populations: the Skykomish River summer Chinook population (which includes the Skykomish, mainstem Snohomish, and Pilchuck River spawning aggregations) and the Snoqualmie River fall Chinook population. The combined Skykomish and Snoqualmie populations comprise the Snohomish River Chinook (Summer/Fall) management unit or "stock", which is one of five in Puget Sound used by the Chinook Technical Committee (CTC) of the Pacific Salmon Commission (PSC) as an escapement indicator for Puget Sound Natural Summer/Fall Fingerlings. The U.S. members of the CTC (USCTC) developed data standards desirable for stock-specific assessments of escapement, terminal runs, and forecasts of abundance against which existing stock assessment programs could be evaluated. The USCTC report found that individual escapement estimates in Puget Sound may range from very good to very poor. The most apparent shortcomings, relative to the USCTC data standards, have been the use of unverified expansion factors primarily for redd surveys, and the absence of variance estimates. This project proposed to address some of these shortcomings in the Snohomish River basin by testing two trans-generational genetic marking methods to estimate adult abundance, genetic mark recapture (tGMR), which estimates census spawner abundance, and rarefaction curve analysis (tRC), which estimates the abundance of successful spawners.

We estimated abundance of Chinook salmon spawning in the Skykomish and Snoqualmie Rivers using genetic-based, closed population, pooled Lincoln-Peterson mark-recapture abundance estimators (tGMR). As the first sampling event, scales and other tissues were taken in the fall 2011 from 149 Chinook adult carcasses (marks) found in the Skykomish River (and tributaries) and from 221 Chinook adult carcasses found in the Snoqualmie River (and tributaries). As the second sampling event, in the spring of 2012, tissues were taken from 1,644 outmigrating juvenile Chinook of natural origin (1,268 from the Skykomish River trap, 376 from the Snoqualmie River trap). Yearling migrants were removed from both juvenile collections and the estimated number of unmarked and untagged hatchery produced juveniles was subtracted from the Skykomish River capture number. Carcass and juvenile tissue samples were genotyped and relationships among parents and offspring (parentage) or among offspring (sibship) were inferred using the genetic data and the statistical algorithms in the software FRANz and COLONY2. Marks were 'recaptured' genetically identifying the fraction of marks determined to be parents of the outmigrating offspring. Counts derived from the relationship information were fed into tGMR or tRC models and abundances were estimated. Final tGMR abundance estimates were expanded to basin-wide totals using redd counts from up- and downstream of the smolt traps.

For Skykomish River estimates, we had genotypes for 87 of the 149 adults, 1,122 of the 1,268 juveniles, and, through parentage analysis, 27 recaptures. For Snoqualmie River estimates, we had genotypes for 136 of the 221 adults, 332 of the 376 juveniles, and, through parentage analysis, 23 recaptures. Of 1,122 Skykomish River juveniles, 19 were identified as yearling migrants and 7.2 were estimated to be unmarked and untagged hatchery produced juveniles resulting in a final capture number of 1,096. Twenty Snoqualmie juveniles were identified as yearling migrants and were removed from analysis. Using these counts, we obtained adjusted estimates of tGMR estimates of Chinook spawner abundance for areas upstream of each smolt trap. Eighty-five percent of redds were found upstream of the Skykomish trap, so the Skykomish River abundance estimate was expanded to include spawning downstream of the smolt trap. No redds were found downstream of the Snoqualmie trap, so no expansion was performed.

System-wide spawner abundance estimates for Skykomish/Snohomish River Chinook were 8,302 (binomial, 95%CI = 5,261-11,343; results from COLONY2) and 6,804 (hypergeometric, 95%CI = 3,352-10,257). System-wide spawner abundance estimates for Snoqualmie River Chinook were 3,542 (binomial, 95%CI = 2,181-4,903, results from COLONY2) and 2,026 (hypergeometric, 95%CI = 1,230-2,822). Performance standards were not met with tGMR methods for 2011 (CV > 15% for all estimates) due to low juvenile trap efficiencies, the low number of carcasses recovered from the record low escapement observed in 2011, and the reliance on scales (vs. fin tissue) from poor quality carcasses that resulted in many samples too few genotyped loci. To increase the probability of meeting the precision

standard, the intensity of spawning ground surveys was increased for brood year 2012 that was commensurate with a significant increase in abundance, fin tissue was collected from all sampled carcasses and smolt trapping effort was significantly increased. Using tRC methods, the estimated BY 2011 N_b of Skykomish River Chinook upstream of the smolt trap was 789 (CSHS, 95%CI = 785-793) and the estimated BY 2011 N_b of Snoqualmie River Chinook upstream of the smolt trap was 311 (CSHS, 95%CI = 308-314).

Snoqualmie River Chinook tGMR abundance estimates were more than 4.5 times greater than the 2011 redd count-based escapement estimate (700) and Skykomish River Chinook tGMR estimates were more than 7 times greater than the 2011 redd count-based spawner abundance estimate (1,180). This discrepancy could be due to violations of assumptions of either method, or that each method estimates a different metric. Any factor that reduces the number of females that dig a redd will increase the disparity between these different types of abundance estimates. Adjustments to capture number were made to meet closed population assumptions, and to the degree that it can be determined, all other assumptions of the tGMR method were met. However, the tGMR estimates rely partly on untestable assumptions, most importantly, the assumption that our carcass collections were unbiased with regard to individual reproductive success, which determines adult capture probabilities in the second sampling event. For example, no adult samples were collected from fish spawning upstream of Sunset Falls in the South Fork Skykomish River. If the average reproductive success of fish spawning in the South Fork Skykomish River were higher than that of fish spawning elsewhere, the tGMR abundance estimates could be biased high. Tissues from adults passed over Sunset Falls were not collected in the fall of 2011 or 2012, but will be collected in the fall of 2013. The hypergeometric model, which may partially ameliorate effects of heterogeneity of capture probabilities, relies heavily on the ability of COLONY2 to correctly infer half-sibling relationships, which needs more research.

The Sentinel Stocks Program provided $217,789 (US dollars) for the Snohomish River Chinook salmon GMR project in FY 2012.

Introduction

Significance to the Pacific Salmon Commission's Sentinel Stocks Program

In Puget Sound, seven Chinook stocks are used as escapement Indicator Stocks by the Chinook Technical Committee (CTC) of the Pacific Salmon Commission (PSC): Nooksack spring, Skagit spring, Skagit summer/fall, Stillaguamish summer/fall, Snohomish summer/fall, Lake Washington summer/fall, and Green River summer/fall. The escapement Indicator Stocks are used to monitor the effectiveness of the management regimes and, if necessary, their status may trigger additional management actions in Aggregate Abundance Based Management (AABM) and Individual Stock Based Management (ISBM) fisheries. The U.S. members of the CTC (USCTC) developed data standards desirable for stock-specific assessments of escapement, terminal runs, and forecasts of abundance against which existing stock assessment programs could be evaluated (USCTC 1997). The USCTC (1997) report found that individual escapement estimates in Puget Sound may range from very good to very poor. The most apparent shortcomings in current escapement estimates, relative to the USCTC data standards, have been the lack of usable age, sex, and length data from surveyed streams, the use of unverified expansion factors primarily for redd surveys, and the absence of variance estimates.

This project proposed to address some of these shortcomings in the Snohomish River basin by testing two trans-generational genetic marking methods to estimate natural spawner abundance. The first method we are testing is parentage-based, trans-generational, genetic mark-recapture (tGMR) to estimate census population size of the spawning population. In tGMR, the first sampling event is sampling from the pool of adults comprised of potential parents in the population. The second sampling event is sampling from the pool of individuals (of any age) comprised of offspring of the parental population. All genotyped adults from the first sampling event are considered "marks", and marks are recaptured when they are genetically inferred as parents of individuals sampled in the second sampling event. Marks, captures (a function of the number of genotyped offspring), and recaptures are fed into a closed population Lincoln-Petersen Mark Recapture model to estimate the census population size of the parental population. For this project, the first sampling event consisted of sampling Chinook adults, post-spawning, as carcasses found on the spawning grounds in the fall of 2011. The second sampling event consisted of sampling outmigrating subyearling juvenile Chinook captured in smolt traps the following spring in 2012.

The second method we are testing is a rarefaction curve method (tRC) to estimate the abundance of successful spawners (the number of effective breeders, N_b). For tRC, a sample is drawn from the pool of individuals comprised of offspring of the parental generation whose abundance is of interest. Offspring are genetically grouped into full- and half-sibling groups and unsampled parents are inferred. Systematic subsamples are taken from the full dataset and the number of unique inferred parents are identified for each subsample. The number of adults that produced the offspring sample is estimated by fitting a mathematical model to the number of unique inferred parents for each subsample. The asymptotic number of unique inferred parents is the estimate of the number of successful breeders. For this project, the offspring sample consisted of outmigrating subyearling juvenile Chinook captured in smolt traps the following spring.

Objectives

The primary objectives of this project are (1) estimate the abundance of Chinook salmon spawners (N) in the Snohomish River Basin using tGMR, and (2) estimate the effective number of breeders (N_b) in the Snohomish River Basin using tRC for brood year (BY) 2011. We propose to meet the bilateral data standards for estimating the number of natural-origin spawners including: 1) individual estimates of spawning escapement should, on average, attain an estimated coefficient of variation (CV) of 15% or less; and 2) those specific estimates shall be demonstrably consistent estimates, that is, methods used to produce them are asymptotically unbiased.

Secondary objectives of this study are (3) to partition the genetic-based abundance estimate for natural spawning Chinook by origin, sex, and age, and (4) to develop a redd expansion calibration factor to adjust historical (or future) redd count-based escapement estimates. Data collected for this project will also serve as a genetic reference collection and may allow the estimation of relative reproductive success of hatchery- and natural-origin spawners.

Methods

Experimental design summary

Closed population Lincoln-Petersen mark recapture methods require two sampling events. In the first, individuals are marked and released. In the second, marked and unmarked individuals are captured or recaptured and enumerated and counts are input into a model to estimate abundance. For the proposed tGMR abundance estimate for Snohomish Chinook, the first sampling event is sampling both natural- and hatchery-origin adult Chinook after they have spawned and died (parents). The second sampling event is sampling outmigrating, natural-origin, subyearling Chinook juveniles (offspring) the following spring. The second sampling event also serves as the only sampling event needed for tRC abundance estimation (though if parents were sampled, they can be included). Carcass and juvenile tissue samples are genotyped and relationships among parents and offspring (parentage) or among offspring (sibship) are inferred using the genetic data. Counts derived from the relationship information are fed into tGMR or tRC models and abundances are estimated. Details of the basic design follow.

Study site

The Snohomish River drains 4,807 square kilometers, emptying into Puget Sound north of Everett, WA (Figure 1). The Snohomish River basin is comprised of two Chinook salmon populations: the Skykomish River summer Chinook population and the Snoqualmie River fall Chinook population. The Skykomish summer population includes spawners in the Pilchuck River, mainstem Snohomish River, the North Fork Skykomish and South Fork Skykomish. Sunset Falls, located at river mile 51.5 on the South Fork Skykomish River just upstream of the confluence of the North and South Forks of the Skykomish, is a natural barrier to upstream migration of anadromous salmonids. However, returning adult Chinook (and other species) are captured in a fish trap downstream of the falls and released upstream of the falls in the South Fork Skykomish each year. The Snohomish fall population includes spawners in the mainstem, Raging River, Tolt River, and Tokul Creek. Of 720 miles of stream and river habitat in the Snohomish basin that support fish, 325 linear stream miles are utilized by Chinook salmon (Haring 2002). The area above Sunset Falls includes 100 miles of habitat available to salmon.

Two Chinook salmon hatcheries are linked directly with the Snohomish system: 1) Bernie Kai-Kai Gobin Salmon Hatchery (hereafter "Tulalip Hatchery") located on the Tulalip Tribal Reservation 3.3 miles north of the mouth of the mainstem Snohomish River, and 2) Wallace River Hatchery, operated by WDFW, located at the confluence of May Creek and river mile (RM) 4.0 of the Wallace River, which enters the mainstem Skykomish River at RM 35.7 (Figure 1). Skykomish-origin summer Chinook broodstock have been used to produce subyearling releases from Tulalip Hatchery since brood year 1998 and both subyearling and yearling releases from Wallace River Hatchery the early 1970's. Fall Chinook releases, which preceded the summer Chinook programs at both hatcheries, were discontinued in brood year 2004 at Tulalip Hatchery and in 1997 at Wallace River Hatchery. Chinook returning to Wallace River Hatchery have provided gametes for both programs for most years since the 1990's. Prior to the 1990's, fall Chinook broodstock of Green River descent were imported as an additional source of broodstock for both the Tulalip and Wallace Hatchery programs and on occasional years prior to 2004 for the Tulalip program. The Wallace River Hatchery program is currently managed as an "integrated" hatchery program, meaning that natural-origin spawners (NOS) are regularly incorporated into the

broodstock for the on-station releases from Wallace River Hatchery. The source of Chinook gametes provided for the Tulalip Hatchery program are considered to be integrated "one generation out" because the brood source comes from the F1 returns that were integrated the previous generation. Natural-origin broodstock are obtained from fish returning to Wallace River Hatchery and a small proportion of fish captured at the Sunset Falls fish trap each year. All (100%) of the Snohomish regional hatchery Chinook production is identifiable through a combination of coded-wire tagging (representative groups), adipose fin clipping (100% less double index tag [DIT] groups), and thermal otolith mass-marking (100% at Tulalip Hatchery, 100% being implemented at Wallace River Hatchery effective brood year 2013).

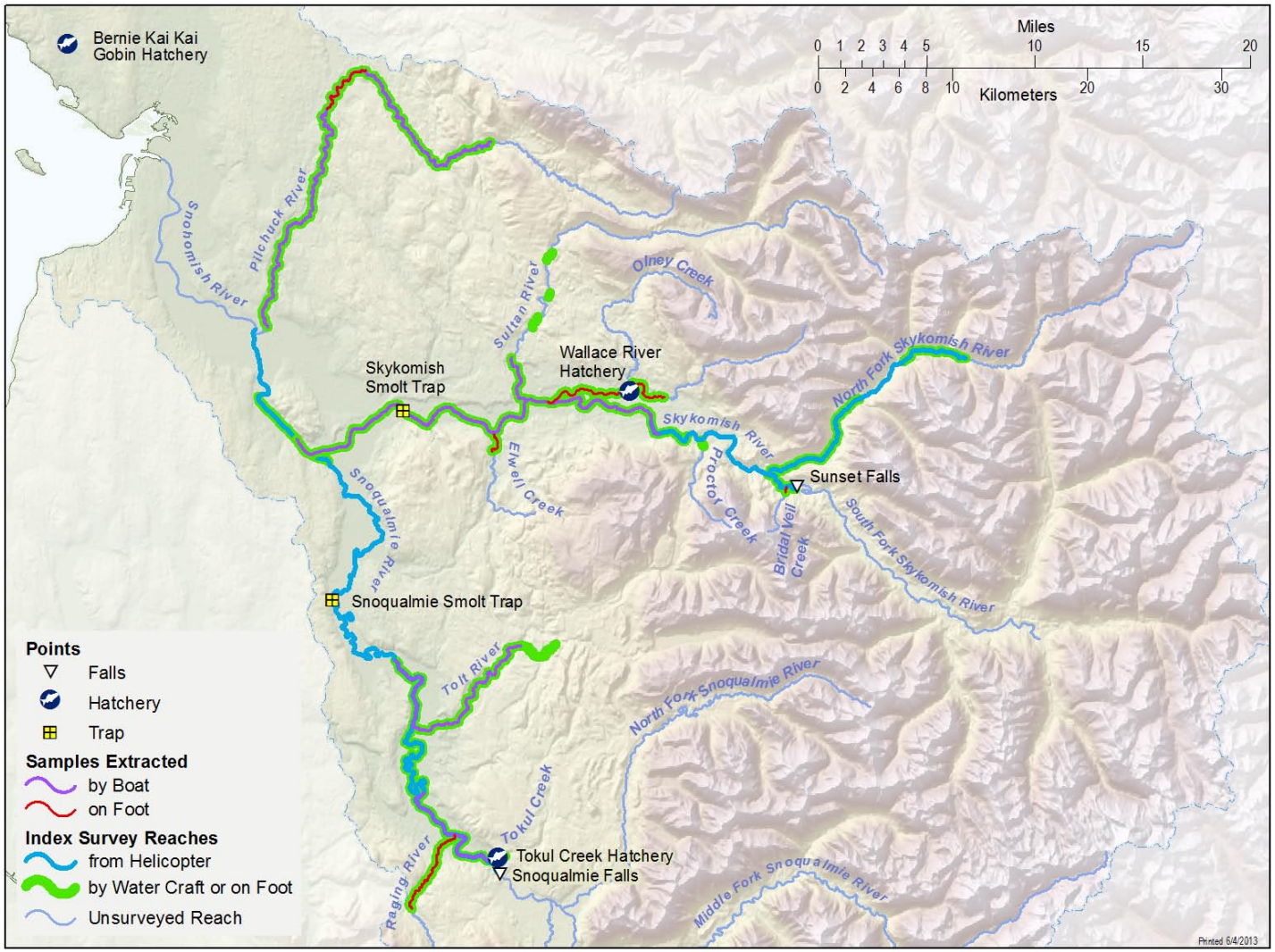

Figure 1. Locations of carcass survey reaches, screw trap sites and hatchery facilities in the Snohomish River basin. Carcass sampling was not performed on the South Fork Skykomish upstream of Sunset Falls in 2011 or 2012. Non-lethal fin tissue sampling for DNA analysis of live adults passed upstream of the falls will begin in 2013.

Field sampling

Carcass Sampling – In the fall of 2011, spawner surveys were conducted throughout the Snohomish basin, from September through November on mainstem and tributary reaches (Figure 1). Redd surveys were conducted by foot, boat/raft, helicopter, and jet sleds and spawned out carcasses biologically sampled every seven to fourteen days. Tissues for DNA analysis, along with three scales (for ageing), two sets of sagittal otoliths (for determination of origin), adipose fin mark status (for determination of origin), CWT status (for determination of origin), sex, and fork and post-orbital hypural (POH) length were collected from every fish, noting the stream reach and date of each fish sampled. Females that appeared to have died before spawning, based on the quantity of eggs retained in their abdomen, were noted. Carcass recovery, as a fraction of spawner abundance, is typically relatively low, ranging from 8-21% and averaging 13% over the past eight years (2005-2012). Thus, to maximize statistical power, tissues (fin) for DNA analysis from all encountered carcasses should be sampled. However, for BY 2011, the first year of the Snohomish River Chinook project, carcass samples were collected for purposes other than abundance estimation. Scales, CWT presence/absence, adipose fin clip status, otoliths, sex, and fork length (FL) were sampled from all interrogated carcasses, but fin tissues were only sampled from natural-origin recruit (NOR) carcasses for the purposes of building the Genetic Analysis of Pacific Salmonids (GAPS) baseline. Thus, scale, tissue, or both types of samples, whichever were available, were used for DNA parentage analysis for this study. Scales were dried on scale cards and fin tissues were preserved in 95% ethanol at ambient temperatures. Genotyping success is typically much higher when tissue samples from high-graded carcasses are used rather than scales from randomly selected carcasses of varying degree of decomposition. In future years (beginning in 2012), fin tissue samples are being collected from all high quality, non-decayed carcasses, regardless of origin.

Juvenile Sampling – In spring of 2012, juvenile Chinook salmon were captured in eight-foot screw traps operated at RM 25 on the Skykomish River and RM 12 on the Snoqualmie River (Figure 1). Trap operations began on February 12, 2012, and continued through June 10, 2012. The traps were fished for three day periods and five night periods each week, except when high flows or excessive debris prevented operation. Each fishing period was 12 hours in length. Captured individuals were netted from the live box and held in five gallon buckets. Fish were placed into a dishpan where they were anesthetized with a solution of clove oil, identified to species, and examined for marks (adipose fin clips, Bismarck Brown dye [used for trap efficiency trials], and CWT). Unmarked/untagged Chinook were measured and FL recorded in millimeters. For DNA parentage analysis, a small piece of caudal fin tissue was collected from all unmarked/untagged subyearling Chinook juveniles and immediately stored in 95% ethanol at ambient temperatures. Unmarked and untagged subyearling Chinook were presumed to be of natural-origin given that all regional hatchery Chinook production is marked through a combination of adipose fin clips and CWTs, less a very small proportion that end up not being marked due to clip and tag loss. For example, adipose fin clip (AD) retention for subyearling summer Chinook released from Wallace River Hatchery in 2011 was 99.3% and 100% for Tulalip Hatchery Chinook while CWT retention rates were 98% for 2011 Wallace River Hatchery subyearling Chinook and 100.0% and 99.9%, for AD+CWT and CWT-Only (DIT group) Tulalip Hatchery subyearling Chinook released in 2011.

The tGMR methods rely on sampling specific cohorts. Yearling juveniles outmigrating in 2012 were produced in BY 2010, a non-target brood year, so any yearlings included in tGMR or tRC analysis would be considered “immigrants”, violating the closed population assumption. Because yearling migrants regularly are found in Snohomish River Chinook, age information was required to distinguish yearlings from subyearlings in fish of borderline size (large subyearlings from small yearlings). All juvenile Chinook < 70 mm FL were assumed to be age-0 subyearlings based on previous findings of Tulalip Tribes’ smolt trap operations (Kubo et al. in preparation). However, scale samples were collected from Chinook exhibiting fork lengths longer than 65 mm in order to validate assignments as age-0 or age-1 outmigrants. Ages of juveniles > 70 mm FL without scale-based age data were inferred using a size at

migration date relationship based on data from scale-aged individuals. Scales were dried on scale cards. Juvenile Chinook were classified through scale analysis as subyearling or yearling migrants by the presence or absence of a freshwater annulus. Individuals with no freshwater annulus (narrowly spaced circuli) prior to ocean entrance were categorized as subyearlings, and individuals with scales exhibiting a freshwater annulus prior to ocean entrance were typed as yearlings (Lance Campbell, WDFW, pers. comm.). All juveniles determined to be one year or older by scale age analysis were removed from further analysis.

Trap efficiency trials were conducted using hatchery-origin subyearling Chinook provided from Wallace River Hatchery. On the Skykomish River, a total of 9,989 adipose fin marked hatchery-origin Chinook were released approximately one mile upstream of the trap over five release periods occurring from March 21 through April 18, 2012). On the Snoqualmie River, a total of 7,987 adipose fin marked hatchery-origin Chinook were released approximately one mile upstream over four release periods occurring March 14 through April 17, 2012. Production estimates were calculated for each week and diel strata. Catch for each diel-weekly strata was calculated by expanding the catch per hour for time fished to the total number of hours in that strata. This assumes a constant catch per unit effort within each strata. The expanded catch was then further expanded by the trap efficiency with a bias correction (Kubo et al. in preparation; K. Finley, Tulalip Tribes, pers. comm.). Trap efficiency was a estimated for the entire season based on the total numbers released and recaptured in each watershed. Total production estimated during the smolt trap operation was summed with extrapolated estimates for migration prior to and following the trapping season.

Genetic laboratory methods

Genomic DNA was extracted from tissue samples using silica membrane kits (Qiagen DNEasy, Valencia CA). Fish were genotyped at the 13 standardized GAPS microsatellite loci (Seeb et al. 2007) plus one additional locus (Ssa197, (O'Reilly et al. 1996); Table 1). Microsatellite alleles were polymerase chain reaction (PCR)-amplified using fluorescently labeled primers. PCRs were conducted in 384 well plates in 5 µl volumes employing 1 µl template with final concentrations of 1.5 mM $MgCl_2$, 200µM of each dNTP, 1X Promega PCR buffer, and 0.05units GoTaq (Promega Corporation) using a "touch-down" protocol. After an initial two minute denature at 94°C, there were three cycles consisting of 94°C for 30 seconds, annealing at 60°C (temperature stepped down 1° each cycle) for 30 seconds, extension at 72°C for 60 seconds. These were followed by 36 cycles consisting of 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 60 seconds, then a final 10-minute extension at 72°C. Samples were run on an ABI 3730xl automated DNA Analyzer and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500LIZ, Applied Biosystems) and GeneMapper software (Applied Biosystems).

Carcass samples, particularly those in more advance states of decomposition, may yield little or poor-quality DNA, which can lead to genotyping errors and incomplete genotypes. Genotyping was critical to the success of the project and genotyping errors had the potential to bias results by preventing assigning of offspring to their true parent(s) of origin. To minimize this type of scoring error, we repeated the PCR for poorly amplifying DNA using lab protocols normally reserved for forensic analysis. If warranted, we also repeated DNA extraction and PCR. Two technicians scored genotypes of all individuals independently and reconciled any scoring differences. Individuals were included in further analysis if they had a minimum of 10 genotyped loci, which allowed for some missing data while providing enough power for accurately assigning parentage. Carcass or juvenile samples genotyped at fewer than 10 loci were excluded from further analysis.

Table 1. Microsatellite loci genotyped in Snohomish River Chinook salmon.

Multiplex	Locus*	Primer conc [uM]	Alleles	Size Range
Ots-M	*Ots*201b	0.35	37	133-342
	*Ots*208b	0.2	30	142-378
	*Ssa*408	0.2	20	180-320
Ots-N	*Ogo*2	0.15	15	200-258
	*Ssa*197	0.25	39	171-318
Ots-O	*Ogo*4	0.18	14	132-170
	*Ots*213	0.18	37	178-378
	*Ots*G474	0.16	11	144-220
Ots-R	*Omm*1080	0.26	41	162-458
	*Ots*3M	0.12	12	122-170
Ots-S	*Ots*212	0.3	27	123-263
	*Ots*9	0.1	6	99-115
Ots-T	*Oki*100	0.37	32	164-353
	*Ots*211	0.2	27	196-337

***Citations for loci can be found in (Seeb et al. 2007) and (O'Reilly et al. 1996).**

To check for systematic scoring issues, we performed a two-tailed exact test of Hardy–Weinberg equilibrium (HWE) for each locus in each adult collection using the Markov Chain method implemented in Genepop 4.1 (dememorization number 1000, 100 batches, 1000 iterations per batch; Raymond and Rousset 1995; Rousset 2008). Significance of probability values were adjusted for multiple tests using false discovery rate (FDR; Verhoeven et al. 2005). F_{IS}, a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of scoring issues, was calculated according to Weir and Cockerham (1984) using Genepop 4.1. Observed and expected heterozygosity was calculated using GDA v1.1 (Lewis and Zaykin 2001).

Parentage and sibship analysis

To assign parents (adult carcasses) to naturally-produced offspring (migrating subyearling Chinook juveniles), we used the likelihood algorithms implemented in the software FRANz (Riester et al. 2009) and COLONY2 (Wang 2004; Wang 2012; Wang 2013; Wang and Santure 2009). Both FRANz and COLONY2 assign parents to offspring using the multilocus genotypes and maximum likelihood methods. We used both programs for two main reasons. First, COLONY2, in addition to parentage assignment, reconstructs the full- and half-sib family structure in a sample of unknowns and infers specific unsampled parents. The algorithms directing the COLONY2 process are computationally demanding, meaning analysis typically takes days and may not converge even after months of analysis. FRANz, on the other hand, only assigns parents to offspring and takes just minutes to produce results. Second, the two statistical methods for tGMR spawner abundance estimation, binomial and hypergeometric (described further below), require slightly different information. Both COLONY2 and FRANz can provide the data needed for the binomial method, but only COLONY2 can provide the data needed for the hypergeometric method (see below for a description of the hypergeometric method).

Parentage analysis was performed separately for Skykomish and Snoqualmie River carcasses and juveniles. Analysis using FRANz was initiated with the estimated genotyping error rate set to 0.01, the maximum number of parents set to 2,000, MCMC iterations for burn-in was 5,000,000, and an additional 30,000,000 iterations for analysis, and the maximum number of mismatching loci set to one. Single-

parent assignments were not accepted if either the assigned parent or the juvenile were genotyped less than 14 loci and there was 1 mismatching locus. COLONY2 was run using the polygamy mating system, without inbreeding, with the probability of parent (mother or father) being in the sampled dataset equal to 0.05. The probability of the parents being in the dataset was derived from the GMR spawner abundance estimate using results of FRANz analysis. In order to evaluate convergence, we ran COLONY2 three times for each dataset with different random number seeds using the 'short run' option, the combined pair-full likelihood method, 'very high' precision of the likelihood, with no allele frequency updating, and no sibship priors. Results of the three runs were compared and evaluated for convergence. If convergence was not reached with short runs, we re-ran COLONY2 analysis with the medium run option. As recommended by the author of COLONY2 (COLONY2 user manual, J. Wang), convergence was achieved when run-to-run variation in results was minimal. We expected minimal variability in parentage assignment of carcasses. However, we anticipated much more variability in the number of inferred, unsampled parents generated through sibship analysis. Thus, we defined minimal variation as less than 5% CV among the three COLONY2 runs in the hypergeometric abundance estimate. If convergence was achieved with short runs, the first short run was used to generate final tGMR and tRC estimates, and the variability among runs was summarized.

Mathematical and statistical models

Transgenerational genetic mark-recapture (tGMR)

To estimate census spawner abundance, we have chosen a closed population "pooled" or "simple" Lincoln-Petersen model (Seber 1982):

$$\widehat{N} = \frac{mc}{r} \quad (1)$$

where *N* is the abundance estimate, *m* is the number of marks, *r* is the number of recaptures, and *c* is the number of captures.

There are two models typically used to estimate spawner abundance and uncertainty using the above equation. The first uses the binomial distribution, which is based on sampling with replacement. We used Bailey's modification of the Lincoln-Petersen model, which corrects bias due to small sample sizes (Seber 1982):

$$\widehat{N} = \frac{m(c+1)}{(r+1)} \quad (2)$$

For this model, *m* is the number of genotyped carcasses, *c* is twice the number of genotyped juveniles (each juvenile is potentially a recapture of its mother and its father), and *r* is the number of carcass parentage assignments made to the genotyped juveniles. Note that all parentage assignments are used, i.e., each instance of the same carcass being assigned to different juveniles is counted. The second model uses the hypergeometric distribution, which is based on sampling without replacement. We used Chapman's modification of the Lincoln-Petersen model, which corrects for bias due to small sample sizes (Seber 1982):

$$\widehat{N} = \frac{(m+1)(c+1)}{(r+1)} \quad (3)$$

For this model, *m* is the number of genotyped carcasses, *c* is the total number of unique parents assigned (sampled, i.e., carcasses) and inferred (unsampled, i.e., inferred by COLONY2) to genotyped juveniles, and *r* is the number of unique carcass parentage assignments made to the genotyped juveniles. Seber's

Goodness of Fit (GOF) test, which is a modification of the *z* test, was used to test the null hypothesis that there was no difference in population estimates (Seber 1982).

Modification of the capture number for both tGMR models for the Skykomish River estimate was necessary due to violation of the closed population assumption (see the Discussion section for a detailed discussion of the assumptions that must be met in order to produce unbiased estimates in these analyses). Any juvenile caught in the trap whose parents were not part of the surveyed spawner population is an "immigrant" using the tGMR methodology. Hatchery-origin recruit (HOR) juveniles, whose parents were spawned in the hatchery, were released upstream of the Skykomish River trap and were regularly captured in the trap toward the last two weeks of the trapping period after their release from Wallace River Hatchery. All HOR Chinook juveniles released from Wallace River Hatchery are marked with an adipose fin clip, a CWT, or both. Not all marking is 100% successful such that a small fraction of HOR juveniles are indistinguishable from NOR juveniles and would be erroneously included in the capture number (adipose fin clip retention was 99.3% and CWT retention was 98% for BY 2011 Wallace River Hatchery subyearling). Since we are interested only in the naturally spawning Chinook abundance, these unmarked HOR juveniles were immigrants. Including them would inflate the capture number causing an upward bias in the abundance estimate. In the models, capture number became an estimated random variable. To estimate the capture number, we calculated the expected number of unmarked/untagged HOR immigrant juveniles $\hat{J}_i$ captured in the smolt trap using the ratio of marked to unmarked juveniles determined in routine "quality control" (QC) adipose fin clip and CWT retention tests done on several hundred Wallace River Hatchery Chinook that are held and examined after marking and tagging is completed annually, and the number of marked HOR juveniles caught in the trap.

$$\hat{J}_i = \frac{H_u J_m}{H_m} \tag{4}$$

Where H_u and H_m are the number of unmarked/untagged juveniles and marked juveniles, respectively, in QC collections of juveniles from hatchery ponds, and J_m is the number of marked juveniles captured in the Skykomish River smolt trap. We did not obtain complete genotypes for all juveniles, so we estimated the number of genotyped immigrant HOR juveniles $\hat{J}_{gi}$ by multiplying the number of genotyped juveniles (J_g) by the proportion of captured juveniles estimated to be unmarked/untagged HOR juveniles

$$\hat{J}_{gi} = J_g \frac{\hat{J}_i}{J_u} \tag{5}$$

where J_u is the number of captured unmarked juveniles. Thus, abundance estimates using Bailey's and Chapman's equations were estimated as

$$\widehat{N} = \frac{m(\hat{c}+1)}{(r+1)} \tag{6}$$

$$\widehat{N} = \frac{(m+1)(\hat{c}+1)}{(r+1)} \tag{7}$$

Where binomial model captures were calculated as

$$\hat{c} = 2\left(J_g - \hat{J}_{gi}\right) \tag{8}$$

and hypergeometric model captures were calculated as

$$\hat{c} = J_g - \hat{\hat{J}}_{gi} \tag{9}$$

The estimated variance of the binomial model abundance estimate, including variance from estimating captures, was calculated using $\hat{c}$ of equation 8 and the following:

$$\widehat{Var}(\hat{N}) = \hat{N}^2 \left(4\widehat{Var}(\hat{J}_{gi}) \left(\frac{1}{(\hat{c}+1)^2} + \frac{(m)^2}{\left(\hat{c}m + \hat{N}\right)^2} \right) + \frac{\hat{c}m\left(\hat{N} - \frac{m}{\hat{N}}\right)}{\left(\hat{c}m + \hat{N}\right)^2} \right) \tag{10}$$

where

$$\widehat{Var}(\hat{J}_{gi}) = \frac{{J_m}^2 \frac{H_u}{H_m}\left(1 + \left(\frac{H_u}{H_m}\right)^2\right)}{n_h} \tag{11}$$

and n_h is the sample size of the collections from the hatchery. The estimated variance of the hypergeometric model abundance estimate, including variance from estimating captures, was calculated using $\hat{c}$ of equation 9 and the following:

$$\widehat{Var}(\hat{N}) = \left(\hat{N} - 1\right)^2 \left(\frac{\widehat{Var}(\hat{J}_{gi})}{(\hat{c}+1)^2} + \frac{\widehat{Var}(r)}{\left(\hat{c}\frac{m}{\hat{N}} + 1\right)^2} \right) \tag{12}$$

where

$$\widehat{Var}(r) = \left[\frac{\hat{c}\frac{m}{\hat{N}}\left(1 - \frac{\hat{c}}{\hat{N}}\right)\left(\hat{N} - m\right)}{\left(\hat{N} - 1\right)} \right] + \left(\frac{m}{\hat{N}}\right)^2 \widehat{Var}(\hat{J}_{gi}) \tag{13}$$

The tGMR estimated spawner abundances initially covered only the spawning grounds upstream of the smolt trap sites. Additional adult Chinook may spawn in mainstem rivers and in tributaries downstream of the smolt trap sites. Any juvenile offspring produced by those adults would have no chance of being captured in the smolt trap and their parents, if sampled and genotyped, would be "emigrants", violating the closed population assumption (see Discussion). For this reason, carcasses found downstream of the smolt trap were not used in this analysis. However, spawner abundance estimates for the entire Skykomish and Snoqualmie River Chinook spawning habitats are required. System-wide total abundance estimates ($\widehat{N_t}$), binomial or hypergeometric model, were generated by expanding tGMR estimates based on seasonal totals of the number of spawners up- and downstream of the smolt trap estimated from redd counts downstream of Sunset Falls and actual counts of fish put upstream of Sunset Falls.

$$\widehat{N_t} = \frac{\hat{N}}{\hat{p}} \tag{14}$$

where

$$\hat{p} = \frac{S_u}{S_t} \tag{15}$$

and S_t is the total number of spawners up- and downstream of the smolt trap, S_u is the number of spawners upstream of the smolt trap, and N_t is the abundance estimate for the entire system. In order to estimate the total number of Chinook redds, spawner surveys were conducted throughout the entire spawning period from September through November in 2011 (Figure 1). Streams were surveyed by foot, boat/raft, or jet sled and spawned out carcasses biologically sampled every seven to fourteen days. Redds were surveyed by helicopter every fourteen to twenty one days. All ground-counted redds were flagged, enumerated and recorded with a GPS waypoint. Helicopter surveys counted total visible redds each successive flight and total redds were estimated using area-under-the-curve methods (Hahn et al. 2007).

Variance of systemwide abundance estimates were estimated using the following equation (Goodman 1960):

$$\widehat{Var}(\hat{N}_t) = \frac{1}{\hat{p}^2}\left[\widehat{Var}(\hat{N}) + \frac{\widehat{Var}(\hat{p})\hat{N}^2}{\hat{p}^2} - \widehat{Var}(\hat{N})\widehat{Var}(\hat{p})\right] \tag{16}$$

where $\hat{p}$ is a proportion with binomial variance and

$$\widehat{Var}(\hat{p}) = \frac{\hat{p}(1-\hat{p})}{n} \tag{17}$$

and n is the number of total number of observed redds.

Rarefaction curve analysis (tRC)

We used the inferred un-sampled and recaptured parent estimates (output from COLONY2) and a rarefaction curve method in order to estimate the number of successful breeders (N_b; cf., Petit and Valiere 2006). Using a bootstrapping and model fitting algorigthm written in R code (R Development Core Team 2010), we sub-sampled juveniles and their inferred or assigned (recaptured) parents at 1% intervals from 1% to 100% of the total number of juveniles plus an additional subsample size of one. At each interval, we sampled from the entire set 10,000 times without replacement, which provided us with 10,000 re-sampled datasets from which to estimate model parameters. To estimate N_b,we fit the re-sampled datasets to the Beverton-Holt model (BH; Beverton and Holt 1956) and the continuous smooth hockey stick model (CSHS; Froese 2008) using the nonlinear least squares method employed by the "nls" command in R. Means and 95% confidence intervals (CIs) were calculated from the distributions of the 10,000 asymptote parameter values estimated from the re-sampled datasets. Each model was evaluated with AIC_c.

Secondary objectives

Estimate the natural spawning Chinook abundance by origin, sex, and age

If no effect of origin, sex or age on reproductive success was detected (see below section "Relative reproductive success of natural- and hatchery-origin spawners"), abundance of naturally-spawning Chinook by origin, sex, and age was estimated by breaking down the tGMR spawner abundance estimate based on the origin, sex, and age distributions of the successfully genotyped adult carcass samples.

Estimate a redd expansion calibration factor

Estimation of a redd expansion calibration factor was not possible with only the BY 2011 tGMR spawner abundance estimate. This objective will be addressed in future reports.

Relative reproductive success of natural- and hatchery-origin spawners

We used a generalized linear model (GLM) to investigate the effects of origin, sex, age, and body length on individual reproductive success. The model was fit using the negative binomial distribution (Anderson et al. 2011) using R.

Results

Sampling

In the fall of 2011, 373 adult Chinook carcasses were sampled from tributaries of the Snohomish basin; 152 carcasses were sampled in the Skykomish River mainstem and surveyed tributaries and 221 carcasses were sampled in the Snoqualmie River mainstem and surveyed tributaries upstream of the smolt trap sites (Table 2). Of these, the majority were collected within the Wallace River, a tributary to the Skykomish River, and Tokul Creek, a tributary to the Snoqualimie River; two streams that are heavily influenced by hatchery production.

Table 2. Numbers of tissue samples collected from adult Chinook salmon carcass recovered in Snohomish River tributaries in the fall of 2011 and numbers processed for tGMR analysis.

Major basin	Stream	WDFW Code	Collected	Laboratory Processed	Comment
Skykomish	Skykomish mainstem	11NB	28	28	
	Bridal Veil Creek	11MW	12	12	
	Elwell Creek	11MX	2	2	
	Olney Creek	11MY	4	4	
	Pilchuck River[a]	11MZ	23	23	
	Proctor Creek	NA	0	0	No carcasses found
	Sultan River	11ND	2	1	One missing scale
	Wallace River	11NG	81	79	Two missing scales
Total			152	149	
Snoqualmie	Snoqualmie mainstem	11NC	62	62	
	Raging River	11NA	20	20	
	Tokul Creek	11NE	109	109	
	Tolt River	11NF	30	30	
Total			221	221	

[a] – The Pilchuck River is located downstream of the Skykomish River smolt trap site, so carcass samples from the Pilchuck River were dropped from tGMR analysis. Abundance of Pilchuck River spawners, and other downstream spawners, were estimated by expanding the tGMR estimate using redd-count based abundance estimates.

Equal numbers of male and female adult Chinook carcasses were sampled in the Skykomish River and tributaries, whereas many more female than male carcasses were sampled in the Snoqualmie River and tributaries in 2011 (Table 3). More HOR than NOR adults were sampled in the Skykomish River basin in 2011, mainly due to the majority of samples being collected from the Wallace River, where, as expected, the average proportion of HORs was higher because the Wallace River Hatchery is

located in that stream. In all other surveyed streams, more NOR than HOR carcasses were sampled, which is consistent with recent (2005-2011) demographic estimates of the origins of hatchery- and natural-origin fish. In the Snoqualmie basin, nearly equal numbers of HOR and NOR carcasses were sampled, which is not consistent with the recent 7-year average HOR and NOR proportions in the Snoqualmie watershed. In addition, sampled HOR and NOR carcasses were not distributed evenly. In Tokul Creek, sampled carcasses were mostly of hatchery origin, which is consistent with past years' proportions. In all other surveyed streams, more NOR than HOR carcasses were sampled, which is also consistent with past demographic-based estimated proportions.

Table 3. Summary of adult Snohomish River Chinook carcasses found upstream of the smolt trap sites by origin and sex.

		Origin			Sex		
Major basin	Stream	HOR	NOR	Unknown	Male	Female	Unknown
Skykomish	Skykomish mainstem	6	22	0	15	13	0
	Bridal Veil Creek	0	12	0	5	7	0
	Elwell Creek	0	2	0	2	0	0
	Olney Creek	3	1	0	1	3	0
	Pilchuck River[a]	10	13	0	11	12	0
	Proctor Creek	0	0	0	0	0	0
	Sultan River	1	1	0	0	1	1
	Wallace River	67	14	0	41	40	0
Total		77	52		64	64	
Snoqualmie	Snoqualmie mainstem	17	42	3	17	45	0
	Raging River	8	11	1	8	12	0
	Tokul Creek	78	31	0	46	63	0
	Tolt River	7	23	0	12	18	0
Total		110	107		83	138	

[a] – The Pilchuck River is located downstream of the Skykomish River smolt trap site, so carcass samples from the Pilchuck River were dropped from tGMR analysis. Abundance of Pilchuck River spawners, and other downstream spawners, were estimated by expanding the tGMR estimate using redd-count based abundance estimates.

The Skykomish River trap was fished for an average of 27% of the daytime hours and 43% of the nighttime hours each week. A total of 1,268 Chinook (0.8% of the total estimated outmigration) were captured (Table 4). Seasonal trap efficiency was estimated for be 2.5% (the range of individual trials was 1.5 to 3.8%). The sub-yearling Chinook catch was expanded to an estimated 107,195 (95% C.I. 90,788 – 123,601) outmigration past the trap during the trapping season. The total production estimate for Chinook upstream of the Skykomish River trap was 146,278 sub-yearlings. Based on a size at migration time relationship of scale-aged fish, 1,196 were estimated to be age 0+ and 19 were estimated to be age 1+ or 2+ (Figure 2). Because fish aged older than 0+ were spawned in years prior to 2011, they were dropped from further analysis. Another 53 were tentatively assigned age 0+, however their age designation was less certain due to the larger size at outmigration earlier in the season (Figure 2). Two of these fish were assigned BY 2011 carcass parents, suggesting that others of similar size might be age 0+ as well. No difference in the proportion of individuals assigned parents in the larger 0+-aged juveniles or in the rest of the juveniles was detected (Fisher's Exact Test, $P = 0.35$). Therefore, these larger 0+ juveniles were included in all further analyses.

Table 4. Weekly Skykomish River juvenile Chinook (WDFW Code 12DB) migration timing and numbers sampled and genotyped.

Statistical Week (2012)	Start Date	End Date	Estimated Total N	Unmarked, untagged sampled N (%)	Genotyped N	% of Weekly Outmigration Successfully Genotyped
1	1-Jan	7-Jan	0	0		
2	8-Jan	14-Jan	434	0		
3	15-Jan	21-Jan	868	0		
4	22-Jan	28-Jan	1,302	0		
5	29-Jan	4-Feb	1,737	0		
6	5-Feb	11-Feb	2,171	0		
7	12-Feb	18-Feb	2,605	0		
8	19-Feb	25-Feb	2,585	0		
9	26-Feb	3-Mar	2,762	0		
10	4-Mar	10-Mar	753	6 (0.004)	5	0.003
11	11-Mar	17-Mar	2,031	16 (0.010)	14	0.010
12	18-Mar	24-Mar	3,139	31 (0.021)	28	0.019
13	25-Mar	31-Mar	8,649	119 (0.080)	107	0.073
14	1-Apr	7-Apr	6,786	118 (0.080)	99	0.067
15	8-Apr	14-Apr	18,874	276 (0.187)	239	0.162
16	15-Apr	21-Apr	34,216	346 (0.235)	306	0.208
17	22-Apr	28-Apr	22,636	0		
18	29-Apr	5-May	3,324	31 (0.021)	29	0.020
19	6-May	12-May	5,252	77 (0.052)	71	0.048
20	13-May	19-May	2,177	81 (0.055)	78	0.053
21	20-May	26-May	4,655	21 (0.014)	21	0.014
22	27-May	2-Jun	4,275	34 (0.023)	31	0.021
23	3-Jun	9-Jun	4,717	49 (0.033)	38	0.026
24	10-Jun	16-Jun	829	60 (0.040)	53	0.036
25	17-Jun	23-Jun	1,250	3 (0.002)	3	0.002
26	24-Jun	30-Jun	3,632	0		
27	1-Jul	7-Jul	2,724	0		
28	8-Jul	14-Jul	1,816	0		
29	15-Jul	21-Jul	908	0		
30	22-Jul	28-Jul	0	0		
Total			147,106	1,268	1,122	

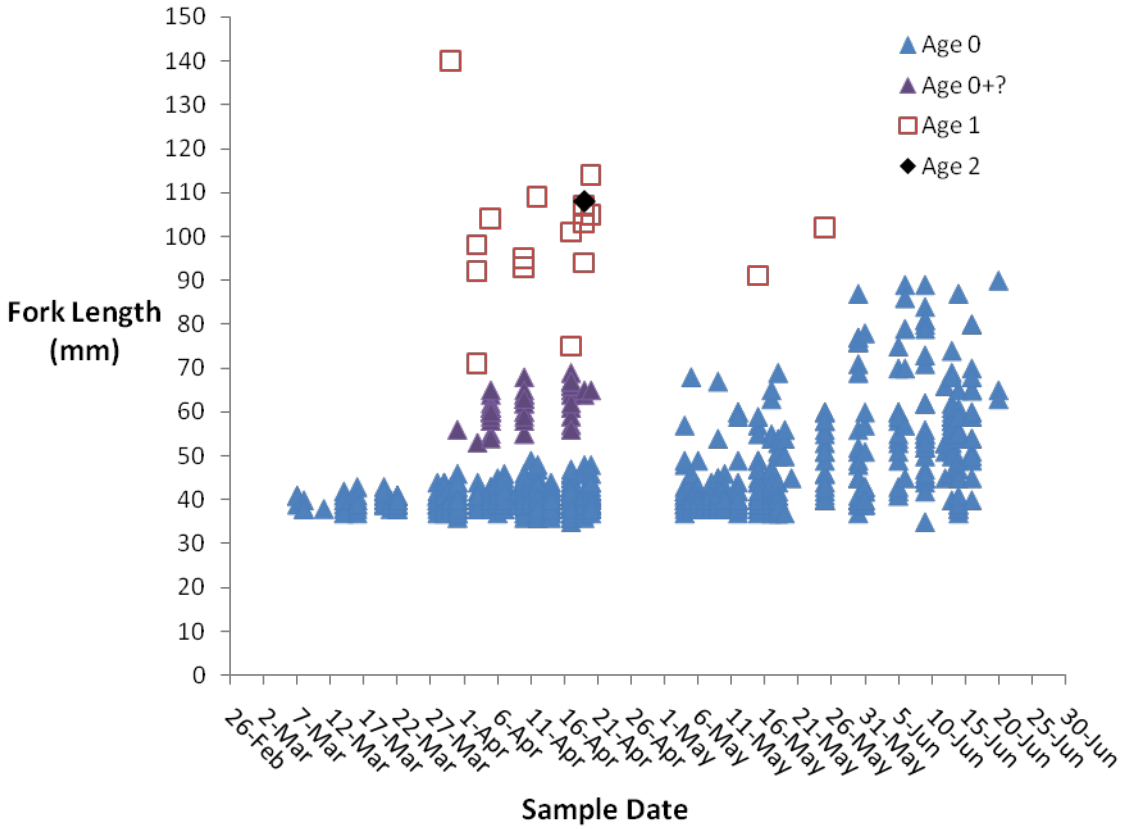

Figure 2. Length at sampling date of juvenile Chinook salmon captured in a smolt trap on the Skykomish River. Most fish > 70 mm were scale-aged due to their larger size, borderlining the size of smaller yearlings that were excluded from the analysis. Open red squares and black filled diamonds represent fish older than age 0+, i.e., fish produced in years previous to 2011. These fish were dropped from further analysis since they originated in non-target brood years. Filled blue triangles represent fish presumed to be age 0+ based on a size at sampling date relationship. Filled purple triangles represent fish whose age was uncertain since they were larger and outmigrated early. Two of these individuals were confirmed as age 0+ by parentage assignment and no difference in the proportion of individuals assigned parents was detected (Fisher's Exact Text, *P* = 0.35). Therefore, the larger 0+? fish were included in further analyses.

The Snoqualmie River trap fished for an average of 24% of the daytime hours and 41% of the nighttime hours each week. A total of 376 Chinook were captured (0.9% of estimated total outmigration). Seasonal trap efficiency was estimated to be 2.5% (range of individual trials was 2.1% to 3.1%). Sub-yearling catch was expanded to an estimated 29,047 (95% C.I. 20,727 – 37,367) outmigration past the trap during the trapping season. The total production estimate for Chinook above the Snoqualmie River trap was 40,632 sub-yearlings (Table 5). Based on a size at migration time relationship of scale-aged fish, 356 were estimated to be age 0+ and 20 were estimated to be age 1+ (Figure 3). Since age 1+ fish were progeny of unsampled adults that spawned in 2010, they were dropped from further analysis.

Table 5. Weekly Snoqualmie River juvenile Chinook (WDFW Code 12DC) migration timing and numbers sampled and genotyped.

Statistical Week (2012)	Start Date	End Date	Estimated Total N	Sampled N (%)	Genotyped N	% of Weekly Outmigration Successfully Genotyped
1	1-Jan	7-Jan	0	0		
2	8-Jan	14-Jan	130	0		
3	15-Jan	21-Jan	260	0		
4	22-Jan	28-Jan	390	0		
5	29-Jan	4-Feb	520	0		
6	5-Feb	11-Feb	650	0		
7	12-Feb	18-Feb	780	0		
8	19-Feb	25-Feb	910	0		
9	26-Feb	3-Mar	928	0		
10	4-Mar	10-Mar	1,615	11 (0.027)	11	0.007
11	11-Mar	17-Mar	566	7 (0.017)	5	0.009
12	18-Mar	24-Mar	261	3 (0.007)	3	0.012
13	25-Mar	31-Mar	1,553	10 (0.024)	10	0.006
14	1-Apr	7-Apr	1,276	16 (0.039)	12	0.009
15	8-Apr	14-Apr	899	6 (0.014)	3	0.003
16	15-Apr	21-Apr	3,398	31 (0.076)	24	0.007
17	22-Apr	28-Apr	2,715			
18	29-Apr	5-May	2,807	32 (0.078)	29	0.010
19	6-May	12-May	5,581	26 (0.063)	25	0.004
20	13-May	19-May	2,062	34 (0.083)	33	0.016
21	20-May	26-May	1,877	34 (0.083)	28	0.015
22	27-May	2-Jun	4,096	29 (0.071)	28	0.007
23	3-Jun	9-Jun	1,664	82 (0.201)	70	0.042
24	10-Jun	16-Jun	1,111	55 (0.135)	54	0.049
25	17-Jun	23-Jun	1,527	11 (0.027)	11	0.007
26	24-Jun	30-Jun	1,222	0		
27	1-Jul	7-Jul	916	0		
28	8-Jul	14-Jul	611	0		
29	15-Jul	21-Jul	306	0		
30	22-Jul	28-Jul	0	0		
Total			40,632	376	335	

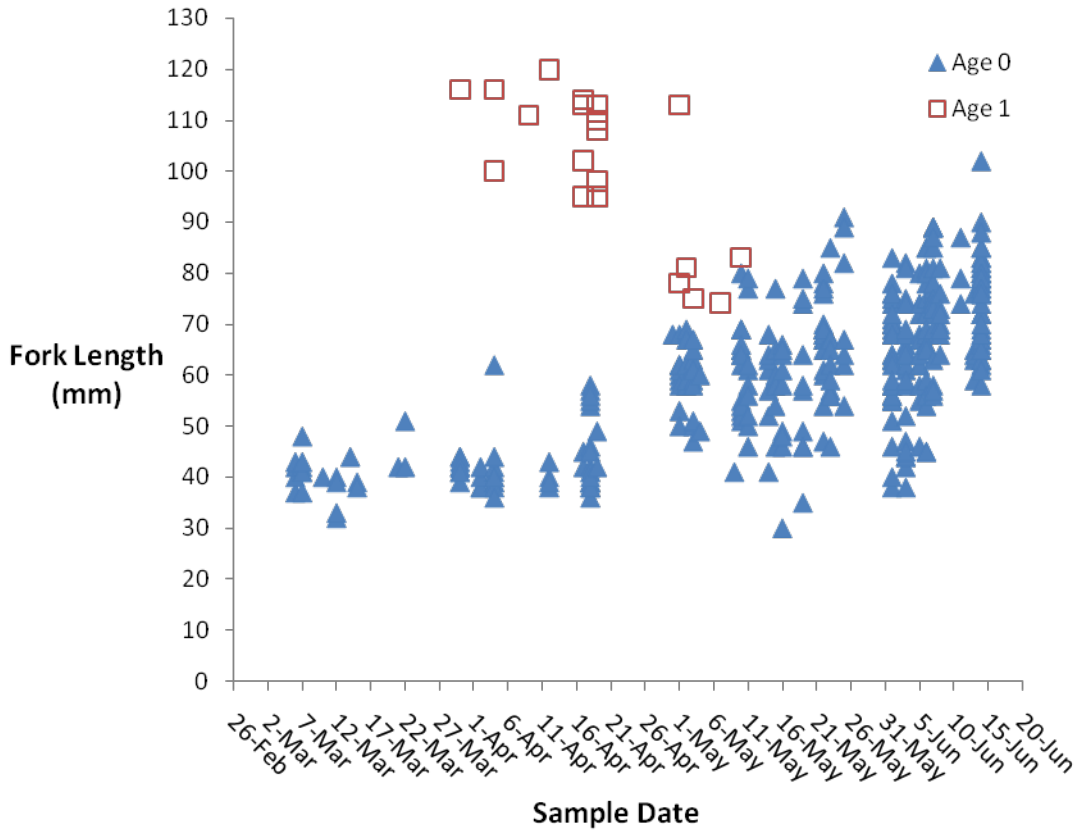

Figure 3. Length at sampling date of juvenile Chinook salmon captured in a smolt trap on the Snoqualmie River. Most fish > 70 mm were scale-aged due to their larger size, borderlining the size of smaller yearlings that were excluded from the analysis. Open red squares represent fish older than age 0+, i.e., fish produced in 2010. These fish were dropped from further analysis. Filled blue triangles are fish inferred to be age 0+ based on a size at sampling date relationship.

Genetics, parentage, and sibship analysis

We were able to obtain usable genotypes (≥ 10 loci genotyped) from 98 of 149 carcass samples from the Skykomish River and from 136 of 221 carcass samples from the Snoqualmie River (Table 6). Genotyping failed or was insufficient for the remaining samples. Within basins and among streams, no difference between the numbers of adult tissues processed and the number with sufficient genotypes was detected (Skykomish, $X^2_{0.05,6} = 3.67$, $P = 0.72$; Snoqualmie, $X^2_{0.05,3} = 0.44$, $P = 0.93$). Within streams and among origins or among sexes, no difference between the numbers of adult tissues processed and the number with sufficient genotypes was detected ($X^2_{0.05,1} > 2.05$, $P > 0.15$ for all tests).

Genetic diversity was high in the Skykomish and Snohomish collections, with average expected heterozygosity = 0.86 and 0.85 for the Skykomish and Snoqualmie, respectively, providing sufficient power for parentage analysis. After correcting for multiple tests, statistically significant deviations from HWE were found at two different loci in each group. F_{IS} values were small for all loci but *Ots*-9 indicating major scoring issues were absent (Table 7, Table 8). Fairly large, positive F_{IS} values were estimated for the Skykomish and Snoqualmie collections for locus *Ots*-9 suggesting a null allele may be present in the populations at this locus. Given the likelihood methods used, parentage assignment should not be affected by the presence of a null allele.

We were able to obtain usable genotypes (≥ 10 loci genotyped) from 1,122 of 1,272 juvenile tissue samples from the Skykomish River and from 332 of 376 juvenile tissue samples from the Snoqualmie River. Genotyping failed or was insufficient for the remaining samples.

Table 6. Genotyped carcass sample sizes for the Skykomish and Snoqualmie Rivers and tributaries upstream of the smolt traps.

Major basin	Stream	Laboratory Processed	Genotyped ≥ 10 Loci	Genotyping Rate (%)
Skykomish	Skykomish mainstem	28	16	57.1
	Bridal Veil Creek	12	11	91.7
	Elwell Creek	2	2	100.0
	Olney Creek	4	1	25.0
	Pilchuck River[a]	23	11	47.8
	Proctor Creek	0	0	0.0
	Sultan River	1	0	0.0
	Wallace River	79	57	72.2
Total		149	98	65.8
Snoqualmie	Snoqualmie mainstem	62	38	61.3
	Raging River	20	15	75.0
	Tokul Creek	109	64	58.7
	Tolt River	30	19	63.3
Total		221	136	61.5

[a] – The Pilchuck River is located downstream of the Skykomish River smolt trap site, so carcass samples from the Pilchuck River were dropped from tGMR analysis. Abundance of Pilchuck River spawners, and other downstream spawners, were estimated by expanding the tGMR estimate using redd-count based abundance estimates.

Table 7. Genetic parameters for adult carcass samples collected in the fall of 2011 in the Skykomish River and tributaries upstream of the Skykomish River smolt trap.

Locus	n	N_A	H_e	H_o	F_{IS}	*P* value[a]
Ogo-2	87	13	0.80	0.82	-0.017	0.579
Ogo-4	87	11	0.76	0.79	-0.043	0.945
Oki-100	51	26	0.95	0.92	0.032	0.345
Omm-1080	82	30	0.95	0.91	0.036	0.572
Ots-201b	87	24	0.95	0.94	0.004	0.351
Ots-208b	86	27	0.95	0.98	-0.026	0.813
Ots-211	84	23	0.94	0.90	0.037	0.186
Ots-212	86	24	0.92	0.91	0.013	0.382
Ots-213	83	28	0.96	0.92	0.042	0.077
Ots-3M	84	10	0.79	0.81	-0.027	0.231
Ots-9	83	5	0.49	0.39	0.209	0.064
Ots-G474	84	10	0.69	0.64	0.074	0.576
Ssa-197	87	31	0.95	0.92	0.035	***0.021***
Ssa-408	87	19	0.91	0.86	0.050	***0.019***
Average	83	20	0.86	0.84		

n = number of individuals genotyped at a locus, N_A = number of alleles, H_e = expected heterozygosity, H_o = observed heterozygosity.

[a]- *P* values in bold were significant at α = 0.05 before correcting for multiple tests and those in bold italics were significant at α = 0.05 after correcting for multiple tests via false discovery rate.

Table 8. Genetic parameters for adult carcass samples collected in the fall of 2011 in the Snoqualmie River and tributaries upstream of the Snoqualmie River smolt trap.

Locus	n	N_A	H_e	H_o	F_{IS}	*P* value[a]
Ogo-2	136	15	0.73	0.68	0.060	0.313
Ogo-4	136	12	0.81	0.80	0.010	0.369
Oki-100	64	24	0.95	0.91	0.044	0.557
Omm-1080	126	39	0.96	0.94	0.018	0.110
Ots-201b	136	33	0.95	0.93	0.021	0.531
Ots-208b	133	29	0.95	0.95	0.001	0.691
Ots-211	131	24	0.94	0.87	0.074	***0.007***
Ots-212	135	28	0.90	0.88	0.017	0.165
Ots-213	130	28	0.95	0.97	-0.025	0.057
Ots-3M	132	8	0.74	0.71	0.032	0.256
Ots-9	128	6	0.46	0.34	0.246	***0.006***
Ots-G474	133	11	0.71	0.70	0.018	0.053
Ssa-197	134	29	0.94	0.96	-0.014	0.888
Ssa-408	135	19	0.90	0.89	0.012	0.543
Average	128	22	0.85	0.82		

n = number of individuals genotyped at a locus, N_A = number of alleles, H_e = expected heterozygosity, H_o = observed heterozygosity.

[a]- *P* values in bold were significant at α = 0.05 before correcting for multiple tests and those in bold italics were significant at α = 0.05 after correcting for multiple tests via false discovery rate.

Skykomish parentage – Maximum likelihood parentage assignment using FRANz identified 28 parentage assignments to 12 individual genotyped carcasses (13.8% of genotyped carcasses). One assigned parent-offspring pair was rejected because the offspring had only 10 loci genotyped and the match was made with one mismatch. All three COLONY2 runs produced identical parentage assignments; 26 parentage assignments were identified to 10 individual genotyped carcasses (11.5% of genotyped carcasses). All parentage assignments were single-parent assignments. The number of unique unsampled parents, inferred through COLONY2 sibship analysis, ranged from 710 to 725 (mean = 718).

Snoqualmie parentage – Maximum likelihood parentage assignment using FRANz identified 26 parentage assignments to 21 individual genotyped carcasses (15.4% of genotyped carcasses). Results of COLONY2 runs varied slightly; two runs produced 23 parentage assignments to 18 carcasses (13.2% of genotyped carcasses) and one run produced 24 parentage assignments to 19 carcasses (14% of genotyped carcasses). All parentage assignments were single-parent assignments. The number of unique unsampled parents, inferred through COLONY2 sibship analysis varied slightly, ranging from 262 to 268.

In both the Skykomish and Snoqualmie datasets, parentage assignments to carcasses made with FRANz or COLONY2 were identical, i.e., for any juvenile, if a parent was assigned by both algorithms it was the same parent. Numbers of parentage assignments differed among algorithms only when FRANz assigned a parent and COLONY2 did not. The number of parentage assignments that differed between COLONY2 and FRANz (five) was not statistically significant than zero (Fisher's Exact Test, $P = 1.000$).

tGMR spawner abundance estimates

Variation among binomial and hypergeometric tGMR estimators based on COLONY2 runs for both the Skykomish and Snoqualmie datasets was minimal (CV < 3%). Thus, for results based on COLONY2 analysis, the final estimate was derived from the first run.

From 25 March, 2012, to 17 June, 2012, 2,488 adipose fin-marked hatchery-produced juveniles were captured in the Skykomish River smolt trap. The marking rate (adipose fin clip and CWT) was high for Wallace River Hatchery releases (98.59%). Thus, the estimated total number of unmarked hatchery-produced juveniles caught in the smolt trap was 9.0. Given genotyping rates, 7.2 were estimated to have been genotyped. Thus, Skykomish River capture numbers for the binomial estimator were reduced by 14.4 (number genotyped times two, see Methods section) and by 7.2 for the hypergeometric estimator.

A total of 361 redds were counted in the Skykomish, Snohomish, and Pilchuck Rivers (Co-manager's annual escapement estimate and carcass survey unpublished data, M. Crewson Tulalip Tribes and P. Verhey WDFW 2012). Of those, 70 (19.3%) were found downstream of the smolt trap and 291 (80.6%) were found upstream of the smolt trap. The estimated number of spawners upstream of the smolt trap, which includes the count of spawners released upstream of Sunset falls, was 1,005 (85.1%). No redds were found downstream of the smolt trap in the Snoqualmie River, so no expansion of the tGMR estimate for the Snoqualmie River population was necessary.

Binomial tGMR estimates of BY 2011 Skykomish River Chinook spawner abundance exceeded 8,000 using parentage data from either FRANz or COLONY2 (Table 9). Binomial tGMR estimates of BY 2011 Snoqualmie River Chinook spawner abundance exceeded 3,000 using FRANz- or COLONY2-based parentage data (Table 10). Hypergeometric tGMR estimates for both systems were less than binomial estimates but were still much higher than those based on redd counts, which were only 1,180 for the Skykomish population and 700 for the Snoqualmie population. Overall Chinook salmon spawner abundance estimates (binomial and hypergeometric) for the entire Snohomish basin were almost three times, to more than seven times, higher than the estimate based on redd counts, which was only 1,880. Coefficients of variation for all tGMR estimates, based on estimated variance, were above the recommended level of 15% (Table 9, Table 10).

Table 9. BY 2011 Skykomish River Chinook tGMR population abundance estimates.

	binomial	binomial	hypergeometric	Redd and trap count
Parentage algorithm	FRANz	COLONY2	COLONY2	
MARKS	87	87	87	
CAPTURES	2191.5	2191.5	722.8	
RECAPTURES	27	26	10	
N - upstream of trap	6813	7065	5790	1005
Var – upstream of trap	1546274.8	1760638.9	2231632.8	
N – system-wide[a]	8006	8764	6804	1180
(95%CI)	(5121-10890)	(5545-11983)	(3352-10257)	
Var – system-wide	2165618.1	2407931.9	3103076.9	
CV (%)- system-wide	18.4	18.7	25.9	

[a]-System-wide estimate includes all production downstream of the Skykomish River smolt trap for the Skykomish Chinook population, which includes the lower Skykomish mainstem, Snohomish mainstem, and lower basin tributaries including the Pilchuck River.

Table 10. BY 2011 Snoqualmie River Chinook tGMR population abundance estimates.

	binomial	binomial	hypergeometric	Redd count
Parentage algorithm	FRANz	COLONY2	COLONY2	
MARKS	136	136	136	
CAPTURES	624	624	280	
RECAPTURES	26	23	18	
N – system-wide[a] (95%CI)	3148 (2007-4289)	3542 (2181-4903)	2026 (1230-2822)	700
Var – system-wide	338667.5	482469.4	164843.9	
CV (%) – system-wide	18.5	19.6	20.0	

[a]-No redds were found downstream of the Snoqualmie River smolt trap, so the population ("system-wide") estimate was based entirely on Chinook production upstream of the Snoqualmie River smolt trap.

tRC estimates of the effective number of breeders (N_b*)*

The effective number of breeders (N_b) for BY 2011 Skykomish River Chinook upstream of the smolt trap, based on the first run of COLONY2, was estimated to be 1,087 (BH, 95%CI = 1,078 – 1,097) or 789 (CSHS, 95%CI = 785-793; Figure 4). Variance among N_b estimates from different COLONY2 runs was minimal (CV < 1.5%). The continuous smooth hockey stick model fit the data better than the Beverton-Holt model (BH average AIC_c = 786.18, 95%CI = 765.51 – 805.78; CSHS average AIC_c score = 688.67, 95%CI = 656.15 – 719.74).

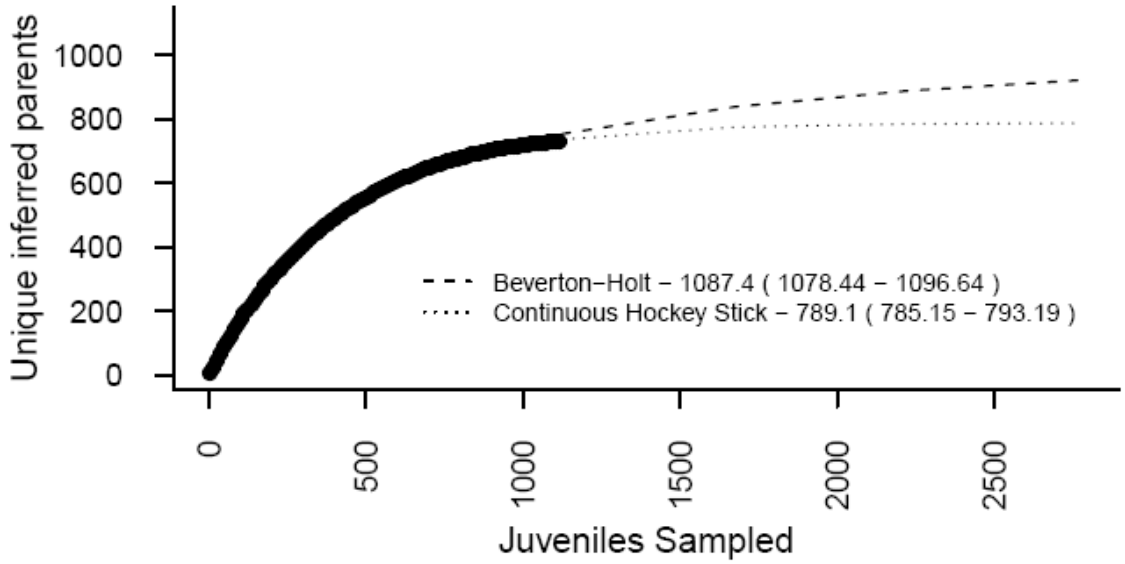

Figure 4. BY 2011 rarefaction curve estimates of the effective number of breeders (N_b) of Skykomish River Chinook, upstream of the Skykomish River smolt trap, based on parentage assigned using COLONY2. Dots represent the means of 10,000 samples of the dataset at each subsample value. Lines represent the values predicted with Beverton-Holt spawner-recruit model (Beverton and Holt 1956) or the continuous smooth hockey stick model (Froese 2008). Lines extending beyond dots were generated using the estimated model and are included to show asymptotic behavior of the models. Values (and their 95%CI) are from 10,000 parameter estimates of the asymptote parameter of each model.

Estimates of the effective number of breeders (N_b) for BY 2011 Snoqualmie River Chinook upstream of the Snoqualmie River smolt trap were 449 (BH, 95%CI = 442 – 455) and 311 (CSHS, 95%CI = 308 – 314; Figure 5). Variance among estimates of N_b derived from different COLONY2 runs was minimal (CV < 2.5%). The continuous smooth hockey stick model fit the data better than the Beverton-Holt model (BH average AIC_c score = 630.27, 95%CI = 606.01 – 653.11; CSHS average AIC_c score = 575.12, 95%CI = 543.69 – 604.37).

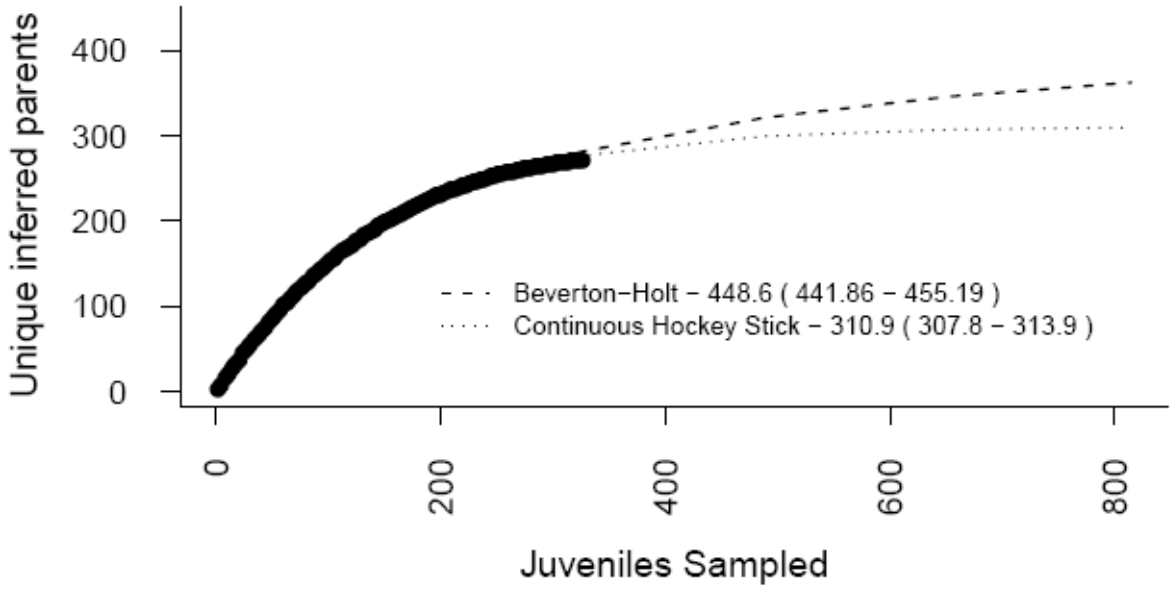

Figure 5. BY 2011 rarefaction curve estimates of the effective number of breeders (Nb) of Snoqualmie River Chinook, upstream of the Snoqualmie River smolt trap, based on parentage assigned using COLONY2. Dots represent the means of 10,000 samples of the dataset at each subsample value. Lines represent the values predicted with Beverton-Holt spawner-recruit model (Beverton and Holt 1956) or the continuous smooth hockey stick model (Froese 2008). Lines extending beyond dots were generated using the estimated model and are included to show asymptotic behavior of the models. Values (and their 95%CI) are from 10,000 parameter estimates of the asymptote parameter of each model.

Secondary objectives

Partition abundance estimates by origin, sex, and age

Too few recaptures were made to reliably partition abundance estimates by origin, sex, and age.

Develop a redd expansion calibration factor

This is the first year of the project. A redd calibration factor will be developed after additional years are completed.

Relative reproductive success of natural- and hatchery-origin spawners

Too few recaptures were made to reliably evaluate the relative reproductive success of natural- and hatchery-origin spawners.

Discussion

Comparison of tGMR and redd count-based abundance estimates

While Rawding et al. (2013) and Small et al. (2012) found much closer alignment between redd based and tGMR-based spawner abundance estimates on the Coweeman and Stillaguamish Rivers, respectively, the Snohomish River tGMR abundance estimates, like the Green River tGMR abundance estimates (Seamons et al. 2012), were much larger than the redd based estimates for the system and for the area upstream of the smolt traps (Table 9, Table 10). A simple explanation for the discrepancy is neither obvious nor intuitive.

One explanation may be that tGMR and redd count-based methods measure different metrics. Mark-recapture estimates, including tGMR, estimate the number of fish at the time of marking (adult sample collection), while redd count-based methods estimate the number of females that constructed redds. Any factor that reduces the number of females that dig redds, for example, pre-spawning mortality (Heard 1991; Keefer et al. 2010; Gilhousen 1990 cited in Quinn 2005), will increase the disparity between the redd based and tGMR estimates. Some female carcasses that, by the presence of a belly full of eggs, are judged to be unspawned are found on the spawning grounds during spawning surveys every year. Eleven (5%) of the female carcasses sampled in the fall of 2011 were judged to be unspawned and pre-spawning mortality is thought to be up to 20% in bad years. However, even 20% is not high enough to account for much of the difference between the tGMR and redd count-based estimates.

Redd count-based estimates may be affected by many problems in redd identification including superimposition, misidentification due to overlap in spawning time with other species (Gallagher and Gallagher 2005), potential for more than one redd per female (Bentzen et al. 2001; Kuligowski et al. 2005), the presence of test digs and variation in redd characteristics (Crisp and Carling 1989), and counting errors due to experience or training of redd counters, and other factors (Dunham et al. 2001; Muhlfeld et al. 2006). While any of these issues may affect the redd count-based estimate, the magnitude and direction of the effect would not be consistent.

The following assumptions must be met in order to produce an unbiased tGMR estimate of abundance (Seber 1982):

1. Genetic marking (as opposed to adipose fin clipping or CWT) will not affect capture probability in the second sampling event,
2. Genetic marks will not be lost before the second sampling event,
3. All genetically marked and unmarked fish are correctly identified and enumerated,
4. The population is closed, and
5. All fish in the population have the same probability of being captured (genetically marked) in the first sampling event OR all fish, genetically marked and unmarked, have the same probability of being captured in the second sampling event.

Violations of these assumptions lead to both upwardly and downwardly biased estimates, and it is not obvious whether the upward or downward biases are stronger.

tGMR Assumption 1 –The probability of capture in the second sampling event is a function of an adult's reproductive success. Adults are sampled after they have spawned and died (or died without spawning). Thus, assumption 1 was met because clipping a fin off a fish that has already spawned and died did not affect the number of offspring it may (or may not) have produced.

tGMR Assumptions 2 and 3 – Using traditional mark recapture methodology, loss of a tag could result in a marked individual being incorrectly identified as unmarked in the second sampling event. Using tGMR methods, a genetically marked adult that failed to produce offspring could be said to have lost its mark. However, instead of resulting in the possibility of that fish being incorrectly identified as unmarked in the second sampling event, 'mark loss' of this type would render recapture of that genetically marked individual impossible. This problem is more correctly associated with tGMR Assumption 4, population closure, and tGMR Assumption 5, identical, independent capture probabilities, discussed below. Alternatively, genotyping or process error that prevents the correct parentage assignment of a genetically marked adult to its captured offspring, while not obviously a 'loss' of a mark,

has the same result as mark loss using traditional mark recapture methods or genetic analogues of traditional mark recapture (Lukacs and Burnham 2005). However, genotyping and process errors seem more suited to tGMR Assumption 3, correct identification and enumeration of marked and unmarked individuals. Thus, for tGMR, we consider assumptions 2 and 3 together, and meeting these assumptions deals with genotyping and parentage assignment errors.

Genotyping error is a common problem for all studies using genetic data, and genotyping errors are typically higher when degraded tissues, such as tissues from carcasses, are used for obtaining DNA (e.g., Copeland et al. 2009). Genotyping errors may lead to parentage assignment errors, and parentage assignment errors may occur in the absence of genotyping errors (Jones and Ardren 2003). If ignored, genotyping and parentage errors will have varying effects on the tGMR estimate depending on the type of error. Erroneously failing to assign a carcass-sampled parent will result in fewer recaptures per capture and an upwardly-biased binomial tGMR estimate or may or may not result in fewer recaptures per capture and a biased hypergeometric tGMR estimate depending on whether or not parentage assignments of the same adult were made to other juveniles. Erroneously assigning the wrong carcass-sampled parent will affect only the hypergeometric estimator, and may cause no bias, or a downward bias, depending on whether or not that parent had also been assigned to other offspring. Erroneously assigning no parent when the true parent was sampled will result in fewer recaptures per capture and an upwardly-biased tGMR estimate. However, due to field and laboratory protocols and parentage algorithms that explicitly account for genotyping error, neither genotyping errors nor parentage assignment errors should bias tGMR abundance estimates. Genotyping errors were minimized first, by high-grading carcasses, taking tissue from only high quality, freshly dead fish, and secondly, by keeping only those genotypes that two laboratory technicians agree upon. This mainly results in the loss of marks due to errors of omission, i.e., our sample size of genetically-marked fish is reduced because many carcass samples are thrown out for lack of complete genotypes (Copeland et al. 2009). Third, any erroneous genotypes that slip through are accounted for by the likelihood methods used to assign parents, which explicitly account for genotyping error (Riester et al. 2009; Wang 2004). Thus, tGMR assumptions 2 and 3 were likely to be met using tGMR methods.

While the assumptions regarding genotyping and parentage were met, parentage assignments were probabilistic with associated uncertainty. The two algorithms used to infer parentage produced slightly different parentage assignment numbers (i.e., recaptures, Table 9 and Table 10). Most parentage assignments were the same. The few that were different involved assignments of a single parent to an offspring. The power to infer single parent assignments is much less than that of inferring two parents simultaneously (Meagher and Thompson 1986), so this result is not unexpected. Ford and Williamson (2010) found parentage was more often assigned to natural-origin ancestry offspring than hatchery-origin ancestry offspring when a statistical threshold was used to infer parentage with statistical confidence. This bias appeared to be caused by a much smaller effective population size in the hatchery stocks. We did not use an arbitrary statistical threshold for assigning parents to offspring, and as a result, our analysis should have been free of this bias. Recapture numbers inferred by FRANz were slightly higher, but not significantly different, than those inferred by COLONY2. The resulting differences in spawner abundance estimates were relatively small (within ~10%), suggesting either software may provide essentially the same estimate, even when the actual parentage assignments are slightly different.

There is greater uncertainty in sibship analysis than in the parentage assignments, which affects hypergeometric estimates. COLONY2 infers unsampled parents based on inferred full- and half-sibling relationships. COLONY2 has been shown to incorrectly split large full-sibling families into multiple smaller full-sibling families (Almudevar and Anderson 2012). Presumably, COLONY2 would identify a large full-sibling family incorrectly split into two full-sibling families as related at the half-sibling level. This would create an extra inferred, unsampled parent, the numbers of which are used in the tGMR hypergeometric model and the tRC N_b abundance estimates, and would bias N and N_b estimates high. However, the size of large families tested by Almudevar and Anderson (2012) was much larger than families the size of those identified through parentage and any family that could plausibly have been sampled. Furthermore, we used the latest version of COLONY2 (v.2.0.4.4), which has been updated to

address this problem (Wang 2013). Still, for a given set of markers, the power to distinguish half-sibling families is much less than the power to identify full-sibling families (Blouin 2003). Incorrectly lumping two unrelated familes at the half-sibling level would produce downward biased numbers of inferred, unsampled parents and downward biased N and N_b estimates. One way to assess the ability of COLONY2 to correctly infer unsampled parentage is to compare the distribution of individual reproductive success of sampled parents (recaptures) to that of inferred, unsampled parents. Too few recaptures were found in BY 2011 Snohomish River Chinook to perform this analysis. However, in a different study, which had more recaptures, the distribution of reproductive success of sampled Chinook parents was different than that of the inferred, unsampled parents (Seamons et al. in preparation), suggesting either the carcass samples were unrepresentative, or COLONY2 did not correctly infer relationships. This ability of COLONY2 to correctly infer unsampled parents needs additional research.

The decision to use a particular parentage algorithm may depend on computation time or whether the binomial or hypergeometric model is preferred. If computation time is important, a binomial estimate could be produced using parentage assigned with FRANz, which takes minutes to complete compared to the days or months needed for COLONY2 analysis. If the hypergeometric model is preferred over the binomial, analysis would have to be done with COLONY2, since COLONY2 is currently the only algorithm that infers half-sibling relationships and inferred, unsampled parents. Parental assignments are probabilistic, but the uncertainty of the inferred pedigree is not propagated through to the abundance estimates. Therefore, our estimates of the variance and CV are also likely biased low.

tGMR Assumption 4 – The assumption of a closed population broadly means that the same individuals available for capture in the first event are available for capture in the second event, i.e., no births, deaths, immigration, or emigration. Using tGMR methods, births and deaths are irrelevant, but "immigration" and "emigration" are possible. Using tGMR methods, an immigrant is any juvenile captured in the smolt trap whose parents were not part of the spawning population of interest. In the Snohomish River system, the main type of immigrants were untagged (no CWT) and unmarked (no adipose fin clip) hatchery origin recruit (HOR) juvenile Chinook presumed to have been released from Wallace River Hatchery directly from the hatchery at RM 4.0 of the Wallace River, a tributary to the Skykomish River beginning at RM 35.7. If left uncorrected, the presence of these HOR juvenile immigrants in our sample would inflate capture numbers and upwardly bias estimates of N. However, in our study, HOR juvenile immigrant effects were eliminated by decreasing capture numbers by the expected number of HOR juvenile immigrants based on QA/QC clip/tag retention rates determined at the hatchery. Correcting capture numbers this way created an additional assumption for the hypergeometric estimator that immigrant HOR juvenile Chinook all came from different full sibling families unrelated at the half sibling level. This assumption may be met since a large number of matings are performed and families are mixed in raceways during hatchery rearing before release. Half-sibling families are created using hatchery spawning protocols (e.g. five females spawned with one male, Wallace River Summer Chinook HGMP; http://wdfw.wa.gov/hatcheries/hgmp/pdf/puget_sound/wallace_summer_chinook_hgmp_final.pdf), so if half-siblings were among the genotyped HOR juveniles, corrected capture numbers and the hypergeometric estimates may be biased slightly low.

Juveniles produced by Chinook adults spawning upstream of Sunset Falls, or in any spawning reaches that were not sampled, could also be considered immigrants (natural origin recruits or NOR progeny of unsampled adults). However, in contrast to HOR juvenile immigrants, NOR immigrants are actually of interest to the abundance estimate, i.e., we want our tGMR abundance estimate to include the parents of all NOR juveniles upstream of the smolt traps. We know of no way to estimate the NOR immigrant fraction of our juvenile collection. However, NOR immigrants should not bias the tGMR abundance estimate if they are not over-represented in the juvenile sample. Over-representation may occur due to chance, but it is more likely to occur if juvenile production is correlated with survey reaches. The quality and quantity of spawning and rearing habitat, and relative productivity and production capacities, are known to vary significantly throughout the Snohomish River basin. If surveyed reaches are correlated with production, tGMR estimates will be biased. For example, if the majority of juvenile

production in the Skykomish River occurs in the South Fork Skykomish upstream of Sunset Falls, where no reaches were surveyed for carcasses, then the majority of fish passing through the trap will be those whose parents were not sampled, upwardly biasing the tGMR abundance estimate (see also discussion of tGMR Assumption 5 below). This is assumed to be an issue for the Skykomish River tGMR abundance estimate since no genetic samples from the South Fork Skykomish River upstream of Sunset Falls were collected in 2011 (tissue sampling of adult Chinook passed upstream of Sunset Falls will begin in the fall of 2013). While juvenile production has not been quantified within survey reaches of the Skykomish River, it is known that the absolute numbers of natural-origin Chinook salmon that have been passed over the Falls in recent years have comprised a significant proportion of the Skykomish Chinook population natural origin spawners (NOS) for the most recent 10 years where this information is available (averaging 30% of the Skykomish Chinook population for 2000, 2001, and 2005-2012, Co-manager's annual escapement estimate and carcass survey unpublished data, M. Crewson Tulalip Tribes and P. Verhey WDFW 2012). NOS Chinook salmon passed over Sunset Falls have averaged approximately 19% of the entire Snohomish basin's natural-origin Chinook production in the most recent (same) 10 years. It is possible that the carcasses of some adults that spawned in unsurveyed reaches, including South Fork Skykomish River, drifted downstream into surveyed reaches and were therefore available for sampling, though this is presumably a rare event. There is no way to distinguish fish that drifted from those that spawned nearby. Thus, there is no way to conclusively determine if our juvenile sample is representative of only the surveyed reaches, but in the case of Sunset Falls, it is highly unlikely given the significant contribution that Chinook production upstream of the falls makes to the Skykomish population.

The presence of NOR immigrants cannot by itself account for the difference between the redd count-based and tGMR abundance estimates. If we assume that the redd count-based abundance estimate is closer to the true *N* and that production is proportional to adult abundance the proportion of sampled, genotyped smolts produced in the South Fork Skykomish was roughly 23% of the total (277 Sunset Falls adults, 1,180 total). If we then reduce the capture number to 1,677 (76% of 2,191.5, Table 9), the binomial tGMR estimate, 6,354, is still more than five times higher than the redd count based abundance estimate, and the 95%CI (4,026-8,682) would still be more than three to seven times higher than the redd count-based estimate. The reproductive success of south fork Skykomish Chinook would need to be roughly eight times higher than the average to account for the difference between redd based and tGMR estimates.

Using tGMR methods, 'emigration' may occur conceptually when an adult fails to spawn (e.g., pre-spawning mortality) or its offspring fail to survive or migrate to the second sampling event, or literally when all of their offspring emigrate before or after the second sampling event. All potential offspring (ova) are not fertilized and offspring mortality occurs after spawning due to, among other things, sedimentation, bed scour, predation, and disease (Quinn 2005). Nevertheless, if the mortality rate is random or equal for sampled and unsampled carcasses, the tGMR estimator provides a consistent estimate at the time of tagging (Seber 1982; Williams et al. 2002).

Genotyped carcasses collected from areas downstream of smolt trap sites could also be considered 'emigrants' since their offspring would never be caught in the smolt trap. Some carcasses collected downstream of the Skykomish River smolt trap were genotyped, but they were removed from tGMR analysis and accounted for by expanding the tGMR estimate based on redd count-based estimates of abundance and the count of fish passed upstream of Sunset Falls. Although the smolt traps are in place for the majority of the outmigration period, it is possible that some families migrate at other times or ages. However, emigration only changes the existing heterogeneity in capture probabilities at the second sampling event, which is the subject of assumption 5.

tGMR Assumption 5 – To meet assumption 5, all individuals must have identical, independent probabilities of capture 1) during the first sampling event, or 2) during the second sampling event (or during both events). Using tGMR methods, assumption 5, part 2 is always violated because the probability of capturing a parent in the form of its offspring is a function of its reproductive success, which, in salmon and trout, is highly variable among individuals (Seamons et al. 2004b; Williamson et al.

2010). This is why emigration, discussed above, does not cause a violation of an assumption; it only shifts or changes the already highly variable capture probabilities.

Violation of part 2 necessitates part 1 be met, i.e., the carcass collection must be representative and unbiased with regard to reproductive success in order to produce an unbiased abundance estimate. While it is impossible to directly test whether or not a carcass collection is representative or biased with regard to reproductive success, we can speculate on possible biases. Too many fish in the carcass collection that "emigrated" or failed to spawn or produce offspring (e.g., prespawning mortalities, Keefer et al. 2010) would mean fewer recaptures per capture biasing the tGMR estimate high. Too many productive fish in the carcass collection would mean more recaptures per capture biasing the tGMR estimate low. The Skykomish River carcass collection may not representative of all spawners since South Fork Skykomish River reaches were not surveyed for carcasses and South Fork Skykomish NOS Chinook comprise a large proportion of the Skykomish escapement. However, as discussed above, any bias due to underrepresentation of South Fork Skykomish carcasses is presumed to be relatively low unless the average reproductive success of South Fork Skykomish adults is much higher than elsewhere in the system. Similarly, if any tributary carcass collection is sampled disproportionately to the average reproductive success, the abundance estimate will be biased. For example, carcasses are easier to find and sample in smaller tributaries, such as the Wallace River or Tokul Creek. If smaller tributaries produce fewer per capita offspring, abundance estimates will be upwardly biased. Under these conditions, the hypergeometric tGMR estimator may be preferred because the heterogeneity in individual capture probabilities is reduced by restricting the offspring per spawner from many to one (Rawding et al. 2013).

tGMR assumption 5 may still be met if the probability of being captured in the first event is independent of the probability of capture in the second event (Schwarz and Taylor 1998). Skykomish and Snoqualmie River carcass collections were likely biased with regard to age and body size (Murdoch et al. 2010; Zhou 2002), and these traits are known to be positively correlated with Chinook reproductive success (Williamson et al. 2010). Thus, it is possible the Snohomish River carcass collections are biased with regard to reproductive success. However, any bias is likely to be small since correlations of body size and reproductive success are typically weak (Dickerson et al. 2005; Rawding et al. 2013; Seamons et al. 2004a; Williamson et al. 2010). Since carcass collections typically consist of larger, older carcasses, unbiased abundance estimates would be even larger than the current estimates, further increasing the difference between the tGMR estimates and redd count based estimates.

Comparison of rarefaction curve (tRC) estimate (N_b*) to tGMR and redd count-based abundance estimates*

Estimates of N_b were much smaller than tGMR spawning escapement estimates, suggesting that roughly 85% of the spawners failed to produce offspring. N_b estimates were slightly closer to the redd count-based estimates (though still three to six times higher), and if we accept the redd count-based estimates as being closer to the true abundance, it suggests roughly 50% of spawners failed to produce offspring. Studies of salmonid reproductive success have found as few as 28% and as many as 75% of spawners failed to produce offspring (Anderson et al. 2010; Ford et al. 2006; Hauser et al. 2011; Hess et al. 2012; Seamons et al. 2004a; Seamons et al. 2007; Williamson et al. 2010). Five years of data for Chinook salmon on Stillaguamish River, which is adjacent to the Snohomish River system, indicated that on average, 58% of spawners failed to produce offspring (range 45% to 78%, Maureen Small, WDFW pers. comm.). Thus, the difference between estimates of N_b and tGMR spawner abundance is larger than expected.

Differences may have been exaggerated by violation of tRC assumptions and downward bias in estimates of N_b. Rarefaction curve analysis to estimate population abundance rests on several assumptions (Eggert et al. 2003; Petit and Valiere 2006):

1. Offspring sample size is sufficient,
2. The population is stable and closed,
3. The capture probability does not vary among individuals,
4. Collections are representative of the population under study, and

5. Families are randomly and independently dispersed.

tRC Assumption 1 – Offspring sample sizes must be large enough to capture the total number of parents that produced them, i.e., they show asymptotic behavior in the rarefaction curve. Thus, needed sample sizes vary with the number of parents that produced them, which is the unknown parameter we are interested in estimating. Additionally, the sample size needed also depends on the distribution of offspring among parents, i.e., very large sample sizes are necessary to have a decent chance of sampling rare families (see also tRC Assumption 3). Thus, target sample sizes need to be large to accommodate a large range of possible N_b values. Power analysis has not been done, but data in figures 4 and 5 appear to be reaching an asymptote. The estimated numbers of unique sampled and inferred parents (unadjusted hypergeometric capture number, 730 – Skykomish River; 280 – Snoqualmie River) were not very different from the estimated asymptotic values estimated through rarefaction (787.3 – Skykomish River; 319.4 – Snoqualmie River), suggesting that the sample size and data were adequate to capture most of the larger families.

tRC Assumption 2 – Like tGMR, rarefaction curve analysis assumes the population of interest is closed, thus immigrants are of concern for tRC analysis. However, since the tRC method does not rely on the carcass sample, and the juvenile sample is assumed to be unbiased with regard to upstream spawning area (tRC Assumption 4), only hatchery-produced immigrants are of any concern. Identification of unmarked and untagged HOR juvenile individuals is impossible. Adjustment using the marking and tagging rates, as was done for the tGMR abundance estimate, is also impossible because individual data is necessary for the tRC method. Thus, no adjustment can be made to the tRC N_b estimate to correct for these immigrants. Any immigrants would likely cause an upward bias in the N_b estimate, though fewer families would mean less bias. Immigrant HOR juveniles are likely to all be from different families, so the bias due to the presence of immigrants is likely to be the maximum possible. The overall effect of the bias is dependent on the number of families in the NOR population. If few families exist in the NOR population, the bias due to HOR immigrants will be large, but if the number of families in the NOR population is the same or more than those in the HOR immigrant population, the bias will be very small or non-existent. Only an estimated 7.2 immigrants were genotyped, less than 10% of the total number of unique inferred parents, so any bias was likely very small.

Emigration of entire families before or after the smolt trapping period would cause a downward bias by reducing the number of families that could be sampled. Both smolt traps are in place throughout most of the emigration period. Reproductive success data from another system suggests that, while there are differences among families in average outmigration date, the within-family variance in outmigration date can be very large, i.e., members of the same full-sibling family were captured in the smolt trap very early and very late in the trapping season (Green River Chinook salmon, Seamons et al. unpublished data). Thus, emigration may add to the already existing heterogeneity in probability of capture and plausibly does not cause significant bias above and beyond that induced by the heterogeneity of capture probabilities (assumption 3).

tRC Assumption 3 – Like tGMR, the tRC method assumes the probability of capture does not vary among individuals. This assumption is violated because of individual variability in reproductive success (see above). Variation in capture probability causes more recaptures of some families and fewer of others than would occur otherwise leading to a downward bias in estimates of N_b. Thus, both the Skykomish and Snoqualmie River N_b estimates are likely biased low.

tRC Assumption 4 – Sampling must be representative of the population under study in order to produce an unbiased estimate of N_b using tRC methodology. Immigrants have already been acknowledged. Our smolt trap collections are more likely to be representative of NOR Chinook spawners upstream of the smolt traps than our carcass collections since we know some spawning reaches were not sampled (e.g., South Fork Skykomish River upstream of Sunset Falls) and all juveniles produced in spawning reaches upstream of the smolt trap must pass by or through the smolt trap.

tRC Assumption 5 – Families must be randomly and independently dispersed as they migrate in order to produce an unbiased estimate of N_b. Any non-random clumping of families during outmigration may lead to an unrepresentative sample and a downwardly biased estimate of N_b. As stated above (under

tRC Assumption 2), differences in family average outmigration date exist, but within family variance is large. The limited parentage assignment results showed that multiple members of the same family were sampled, but whether or not this differs from random expectations is unknown and the number of assignments is too small for statistical testing.

tRC Additional concerns – The tRC estimate of N_b is for upstream of the smolt traps only. One could expand N_b estimates based on redd count-based abundance estimates, similar to the expansion of the tGMR abundance estimate. Such an expansion would create an additional assumption that reproductive success of spawners downstream of the smolt traps is distributed the same as spawners upstream of the traps. There is no *a priori* reason to believe that individual reproductive success of fish spawning in reaches downstream of the smolt traps was any different than those spawning upstream of the smolt traps. The added uncertainty would also need to be incorporated.

Project summary and conclusions

Carcass collection and sampling were performed and completed according to schedule and there were no delays or problems with redd surveys in the fall of 2011. In the spring of 2012, smolt trapping was conducted, biological samples were collected and juvenile abundance estimates were estimated and provided to WDFW by the Tulalip Tribes on schedule.

Genotyping of adult carcass tissues was delayed due to extensively degraded carcass tissues of mainly HOR samples. From most HOR carcasses, only scales were sampled for ageing analysis. Fin tissue collection were focused solely on NOR carcasses for use in updating the GAPS genetic baseline. Degraded and poor quality genotypes need to be processed more than once to obtain usable genotypes, producing more scheduling delays than were anticipated. Such delays should not occur in year two. In the fall of 2012, operculum samples, in addition to scales, were collected from natural- and hatchery-origin Chinook that were high-graded for quality. Juvenile tissue genotyping was also delayed. Any yearling juvenile migrants encountered in the 2012 outmigration were by definition produced prior to the first year of the Snohomish tGMR project that began with brood year 2011 adults. Our intention was to have all juveniles aged prior to genotyping so that we did not genotype fish that would not be used. Aging took longer than expected. Ultimately, the decision was made to genotype all juveniles and then remove the yearling migrants from the analysis afterward. Part of the basis for this decision was the fact that the number of juveniles actually sampled was far fewer than the number expected and budgeted. Therefore, no proportional sub-sampling was necessary and all juvenile samples could be genotyped. Juvenile genotyping delays also will not occur in the second year of the project because yearling and subyearling migrants can now be used to inform tGMR analysis. Yearling juveniles outmigrating in 2013 were produced in BY 2011. BY 2011 tGMR analysis can be augmented and reanalyzed with yearling juveniles that migrate in 2013.

Because genotyping was delayed, all subsequent analyses were delayed. For this reason, preliminary estimates were not available in late-November of 2012. By the time genotypes were available and preliminary estimates could be made, regional WDFW biologists and co-managers were heavily involved in the North of Falcon process and were not available to review the tGMR estimates. The executive summary and the final progress report were delayed for the same reason. If genotyping occurs on time in year two of the project, preliminary estimates will be made early enough that regional biologists and co-managers will have time to review them before getting caught up in the annual management process.

In 2011-12, we implemented the first tGMR study in the Snohomish system to estimate Chinook salmon abundance. We modified the basic tGMR study design to account for unmarked hatchery releases, age-1 smolts, and expanded for production downstream of the trap sites. Our tGMR estimates were 3-7 times the traditional estimate based on redd surveys and hard counts of adults passed over Sunset Falls. In 2011, we sampled few carcass, due in part to the record low estimated escapement and in 2012, we sampled low numbers of migrant juveniles, due to the record low escapement exacerbated by

very low juvenile trap efficiency, which led to low precision (CV range 18-26%) for the escapement estimates. Estimated Chinook abundance was much higher in the fall of 2012, which along with greatly improved tissue quality and increased smolt trapping effort, will help to address issues with precision and bias in the tGMR estimate. Some concerns remain with the equal probability of capture assumption and WDFW is pursuing simulations and exploring alternate models to better address this concern.

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