Progress Report

Spawner abundance estimates for Green River Chinook salmon

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Executive Summary

The Green River fall Chinook salmon population 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 Green 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 Green River 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 382 Chinook adult carcasses (marks) found in the Green River and Newaukum Creek. As the second sampling event, in the spring of 2012, tissues were taken from 2,548 outmigrating juvenile Chinook of natural origin. The estimated number of unmarked and untagged hatchery produced juveniles was subtracted from the juvenile capture number. Tissue samples from carcasses and a representative sub-sample of juveniles (n = 1,996) were genotyped at 14 microsatellite loci 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.

We obtained genotypes for 328 of the 382 adults, 1,902 of the 2,548 juveniles, and, through parentage analysis, 396 recaptures. Of 1,902 genotyped Green River juveniles, 24 were estimated to be unmarked and untagged hatchery produced juveniles resulting in a final genotyped juvenile number of 1,878. Using these counts, we obtained adjusted estimates of tGMR estimates of Chinook spawner abundance for areas upstream of the smolt trap. Ninety-eight percent of redds were found upstream of the Green River trap, so the Green River abundance estimate was expanded to include spawning downstream of the smolt trap. The system-wide spawner abundance estimates for Green River Chinook was 3,201 (binomial, 95%CI = 2,778-3,671; results from COLONY2) and 2,868 (hypergeometric, 95%CI = 2,493-3,321). Performance standards were met with tGMR methods for 2011 (CV = 7% for all estimates). Using tRC methods, the estimated BY 2011 N_b of Green River Chinook upstream of the smolt trap was 1,003 (continuous smooth hockey stick model, 95%CI = 785-793).

Green River Chinook tGMR abundance estimates were approximately three times greater than the 2011 redd count-based escapement estimate (993). 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. The 1,689 surplus adults released by the Muckleshoot Tribe were included and were estimated in the tGMR abundance estimates, but were not completely counted in the redd count-based estimate due to an apparently high rate of pre-spawning mortality.

To the degree that it can be determined, all assumptions of the tGMR method were met, except the closed population assumption. Adjustments to capture number for unmarked hatchery origin juveniles were made to meet closed population assumptions. However, natural production of juvenile Chinook in Soos Creek is substantial, and because the number of these fish that were likely caught in the trap was unknown, no adjustment was made, and the tGMR abundance estimate is likely upwardly biased by some unknown amount. In addition, the tGMR estimates relied on untestable assumptions, most

importantly, the assumption that the carcass collection was unbiased with regard to individual reproductive success, which determines adult capture probabilities in the second sampling event. 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.

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 Green 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 Green River Basin using tGMR, and (2) estimate the effective number of breeders (N_b) in the Green 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 evaluate the feasibility and efficacy of a

factor for adjusting 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 Green River 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 some 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 Green River drains 1250 square kilometers, emptying into the Green-Duwamish River before reaching saltwater in Elliott Bay, Puget Sound, through the shipyards of Seattle (Figure 1). Its mainstem flows are regulated at Howard Hanson Dam (rkm 104) and at the City of Tacoma's municipal water supply diversion dam ("Tacoma Headworks Trap"; rkm 98). The gradient is low and the substrate is composed primarily of sand and silt in the lower 40 km. Extensive diking confines the channel and flood plain in this area. From rkm 40 to 73 the gradient and channel complexity increase, which lead to suitable salmon spawning substrate. Upstream of rkm 73 through 94, the river flows through a gorge extending almost to Palmer, WA. This area contains pockets of spawning gravel but is dominated by bedrock and boulder substrates. The 5km of river upstream from Palmer to the diversion dam is characterized by substrates composed largely of boulders and cobble interspersed with pockets of gravel. Substantial runs of Chinook, chum, pink, and coho salmon as well as smaller populations of steelhead, cutthroat trout, and sockeye salmon are present in the Green River system. Chinook salmon spawn in mainstem areas and the two larger tributaries, Soos and Newaukum creeks, which join the mainstem at rkm 54 and 66, respectively. Chinook spawn in the Green River from rkm 40 near Kent to the Tacoma Headworks diversion dam at rkm 98. This diversion dam and the larger Howard Hanson Dam (found upstream) are impassable barriers to adult salmonid migration.

Green River Chinook salmon are listed as "Threatened" under the federal Endangered Species Act and belong to the Puget Sound Chinook Major Ancestral Lineage (MAL). Within this MAL, Green River Chinook salmon are one of ten stocks that belong to the South Puget Sound, Hood Canal and Snohomish Summer and Fall genetic diversity unit (GDU). This Chinook population is comprised of hatchery origin recruits (HOR) and natural origin recruits (NOR), and about 60% of the naturally spawning fish are of hatchery origin. Two salmon hatcheries operate within the Green River system: Soos Creek Hatchery (WDFW) and Keta Creek hatchery (Muckleshoot Indian Tribe). The Chinook salmon broodstock for these two associated programs has come from natural Green River stock since the early 1900s (WDFW, 2002). Adult Chinook returning to Soos Creek Hatchery are spawned and the eggs incubated briefly before a portion is transferred to the Keta Creek Hatchery for Tribal rearing and release. The remainder of the eggs is incubated at Soos Creek Hatchery, and some are then transferred to the Icy Creek facility for release as larger yearlings. Fish reared at Keta Creek Hatchery are transferred to Palmer

Ponds rearing facilities for further rearing and release. Currently, at both hatcheries all fingerlings released annually are adipose fin (AD) clipped and a portion are coded wire-tagged (CWTed).

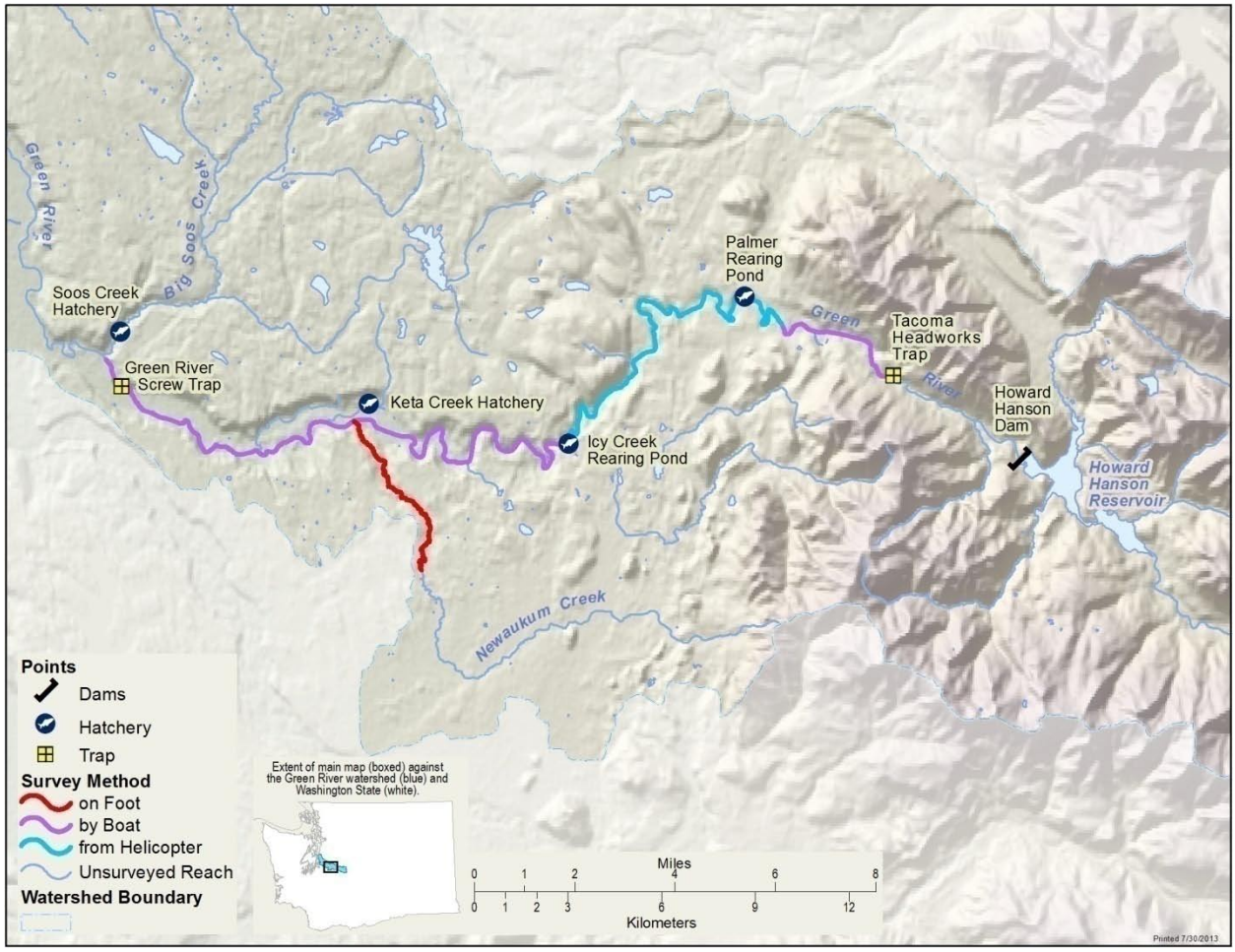

Figure 1. Locations of carcass survey reaches, screw trap and hatchery facilities in the Green River. Carcass sampling was not performed on the canyon reach between Palmer Rearing Ponds and Icy Creek Rearing Ponds due to dangerous conditions and minimal probability of retrieving carcasses.

Field sampling

Carcass Sampling – In the fall of 2011, spawner surveys were conducted throughout the Green River basin, from September through November on mainstem and tributary reaches (Figure 1) with a goal of inspecting 20% of the spawners in natural spawning areas. The target stretch of river was from rkm 42 to rkm 98, broken into four sections. Each section was surveyed at least once weekly. The date, GPS location, sex, fork length/postorbital-hypural length, and the origin (hatchery or wild) based on the adipose fin condition (missing adipose fin = hatchery origin, adipose intact = natural origin) and CWT presence (present = hatchery origin regardless of adipose fin condition) was recorded for each carcass encountered. Scales and fin tissue were collected from all fish; fin tissue was stored immediately in 95% ethanol at ambient temperatures. Following sampling, tails were cut off at the caudal peduncle to assure that re-sampling would not occur, and the carcasses were released into the stream. Redds were counted in various sections of the Green River and Newaukum Creek throughout the spawning season. A

systemwide redd count (excluding Newaukum Creek due to tree cover) was conducted by helicopter near peak spawning at statistical week 40 (October).

Juvenile Sampling – In spring of 2012, juvenile Chinook salmon were captured in a floating five-foot screw trap located on the left bank at river mile 34.5(rkm 55, Figure 1; Topping and Zimmerman 2013). The trap was checked for fish at dawn and dusk each day and at additional times when required by heavy debris loads or large catches. Captured fish were anesthetized with tricaine methanesulfonate (MS-222), identified to species and enumerated. Marking status (adipose fin clips or Bismarck Brown dye [used for trap efficiency trials]) was recorded for each fish. Approximately 50% of the juvenile Chinook captures each week were sampled for genetic analysis. The remainder of the catch was used for trap efficiency trials. 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 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.

Subsampling of juvenile outmigrant collections for genotyping was conducted proportional to the total estimated number of outmigrant Chinook. In order to estimate total outmigration, daily trap efficiency trials were conducted for Chinook with NOR fish captured for the first time and marked with either Bismarck Brown dye or the fin clip taken for DNA analysis (Topping and Zimmerman 2013). Marked fish were released at dusk into fast flowing water 150 m upstream of the trap, upstream of a bend in the river, after being allowed to recover in fresh water. The release site was selected to maximize mixing of marked and unmarked fish while minimizing in-river predation. Bismarck Brown dyed or caudal fin clipped fish caught in the trap were recorded as recaptures. Freshwater production (total Chinook juvenile migrants) was estimated using a single partial-capture trap design, fully described in Volkhardt et al. (2007), and more specifically for the Green River in Topping and Zimmerman (2013). Briefly, the approach estimates missed catch, efficiency strata, time-stratified abundance, extrapolated migration outside the trapping season, and total migrant abundance.

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 (*Ssa*197, 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 Green 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).

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 at 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.

COLONY2 infers unsampled parents through sibship analysis. To evaluate the ability of the COLONY2 algorithm to correctly infer unsampled parents, the distribution of reproductive success of sampled parents was compared to the reproductive success distribution of inferred unsampled parents using the Kolmogorov-Smirnov *D* statistic and a permutation test. Data were permuted 100,000 times.

Reproductive success may differ between spawners in Newaukum Creek and Green River, violating the equal catchability assumption of the tGMR model (see Discussion section). Differences in the mean and distribution of reproductive success were compared between Newaukum Creek and Green River carcasses using a permutation test of Kolmogorov-Smirnov *D* statistic (distributions) and the *z* statistic (means). Data were permuted 100,000 times.

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 methods. The first uses binomial distribution, which is based on sampling with replacement. This equates to using all genotyped juveniles even if they originated from the same parents. One benefit of this approach is that it uses all the available data.

$$r \sim \text{Bin}(c, p) \quad (2)$$

$$m \sim \text{Bin}(N, p) \quad (3)$$

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.

$$r \sim \text{Hypergeometric}(m, c, N) \quad (4)$$

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.

Modification of the capture number for both tGMR models 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. From the start of juvenile trapping in statistical week 5 through week 17, there were no adipose fin-clipped Chinook salmon. We had expected this to be the case for the entire season, but a few hatchery-produced fish were observed beginning in week 18 and we continued to trap them through the end of the trapping season. Some of these fish were not from an intentional release but escaped from the Keta Creek Hatchery or Palmer Rearing Ponds prior to the scheduled release, which occurred after trapping was completed. The cause is unknown but likely due to leakage of water and fish from the hatchery facilities. Adipose clipped parr migrants trapped later in the season were likely from fish intentionally released starting week 22 from Soos Creek Hatchery, located downstream of the trapping site (see below). These fish likely swam upstream from the mouth of Soos Creek and were subsequently captured in the smolt trap.

All HOR Chinook juveniles released from Soos Creek Hatchery and Palmer Rearing Ponds were marked with an adipose fin clip, a CWT, or both in 2012. Not all marking is 100% successful such that a small fraction of HOR juveniles were indistinguishable from NOR juveniles and would be erroneously included in the capture number (adipose fin clip retention was 92.3%for BY 2011 subyearling Chinook released from Palmer Rearing Ponds and 98.2% from Soos Creek Hatchery). Since we are only interested in the naturally spawning Chinook abundance, these unmarked HOR juveniles were 'immigrants' using tGMR methodology. Including them would inflate the capture number causing an upward bias in the abundance estimate.

The BY 2010 Green River Chinook tGMR estimate was corrected for hatchery releases from Icy Creek Ponds. However, it is now understood that only yearling fish, which are easily visually distinguished from subyearling fish by their large size, are released from Icy Creek facilities. In addition, the 2010 estimate was not corrected for releases from Soos Creek Hatchery. At the time, it was believed that juveniles released from Soos Creek Hatchery, which is downstream of the smolt trap, were not swimming upstream and being caught in the smolt trap. Adipose fin-clipped juveniles were captured in the smolt trap in years when no hatchery fish were released upstream of the smolt trap (Topping and Zimmerman 2011, Pete Topping, WDFW, unpublished data) indicating that fish released from Soos Creek Hatchery may indeed swim upstream and end up captured in the smolt trap. Therefore, corrections were made for Palmer Ponds and Soos Creek Hatchery juveniles.

To correct capture numbers for HOR immigrants, we used the hatchery QA/QC adipose fin clip rates to modify the number of captures by the estimated number of HOR immigrants. Hatchery QA/QC marking rates were estimated by subsampling hatchery-produced juveniles at both Soos Creek (n = 1000) and Palmer (n = 2000) hatchery rearing ponds and counting the number of fish with or without an adipose fin. Fish reared at Palmer Ponds cannot be distinguished from those from Soos Creek Hatchery. Therefore, the assumption was made that all adipose fin-clipped juveniles caught in the trap prior to the release of fish from Soos Creek Hatchery, which started the week of 21 May (statistical week 22), originated from Palmer Ponds. All adipose fin-clipped fish trapped week 22 through the end of trapping

(week 29) were assumed to originate from Soos Creek Hatchery. A small fraction (6.6%) of the juveniles released from Soos Creek Hatchery is intentionally left with adipose fins intact as a Double Index Tag (DIT) group. These fish were visually indistinguishable from NOR juveniles at the smolt trap. However, based on the number of clipped fish captured in the smolt trap, the expected number of DIT fish passing through the trap was less than one individual, so no correction was made for DIT fish.

To simultaneously estimate the number of genotyped unmarked HOR juveniles and the uncertainty in that estimate, adjustments to the spawner abundance estimate were made using Bayesian methods. From the subsamples from Soos Creek Hatchery and Palmer Rearing Ponds, we obtained the number of unclipped and clipped juveniles in each facility. The marking rate was modeled as

$$MarkPresent_i \sim \text{Bin}(HatchMarkRate_i, SampleSize_i) \quad (5)$$

where *MarkPresent* is the number of adipose fin-clipped juveniles in the subsample and *SampleSize* is the number of fish in the subsample from each of the two hatchery facilities (*i*). This was done separately for Palmer Ponds and Soos Creek Hatchery. The expected weekly total number of hatchery fish (marked and unmarked) sampled by the smolt trap was calculated by dividing the number of marked fish caught in the trap each week by the estimated mark rate.

$$HORJuves_{ij} = \frac{MarkedSampled_{ij}}{HatchMarkRate_i} \quad (6)$$

The expected weekly number of immigrant (unmarked) hatchery fish sampled by the smolt trap was calculated by subtracting the number of marked fish caught in the trap each week (*j*) from the estimated weekly total number of hatchery fish sampled by the trap.

$$Immigrants_{ij} = HORJuves_{ij} - MarkedSampled_{ij} \quad (7)$$

Juveniles sampled at the trap were subsampled for genotyping and sufficient genotypes were not obtained from all subsampled tissues, so the weekly number of genotyped immigrant juveniles was estimated by multiplying the weekly number of sampled immigrants by the weekly fraction of juveniles successfully genotyped.

$$GenImmigrants_{ij} = Immigrants_{ij} * GenRate_j \quad (8)$$

The adjusted capture number for the binomial estimator was obtained by subtracting the sum of the weekly genotyped immigrants from the total number of genotyped unmarked juveniles and multiplying by two.

$$AdjBinCapture = 2\left(GenUnmarked - \sum GenImmigrants_{ij}\right) \quad (9)$$

The adjusted capture number for the hypergeometric estimator was obtained by subtracting the sum of the weekly genotyped immigrants from the number of unique parents assigned (recaptured) and inferred by COLONY2.

$$AdjHypCapture = UniqueParents - \sum GenImmigrants_{ij} \quad (10)$$

For the binomial estimate, the proportion of recaptured genotypes was modeled as:

$$BinRecap \sim \text{Bin}(AdjBinCapture, markRate) \quad (11)$$

and the abundance of naturally-spawning adults (*BinN*) upstream of the trap was:

$$marks \sim \text{Bin}(BinN, markRate) \quad (12)$$

where *BinRecaps* is the total number of genotyped carcasses assigned to juveniles (recaptures), and *marks* is the total number of genotyped carcasses. For the hypergeometric estimate, the proportion of unique recaptured genotypes was modeled as

$$HypRecap \sim \text{Hypergeometric}(marks, AdjHypCapture, HypN) \quad (13)$$

using the probability distribution function in WinBUGS (see Appendix, Lunn et al. 2000).

The tGMR estimated spawner abundances initially covered only the spawning grounds upstream of the smolt trap site. Additional adult Chinook may spawn in the mainstem river downstream of the smolt trap site. Any juvenile offspring produced by those adults may 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 the Discussion section for more detail). For this reason, carcasses found downstream of the smolt trap were not used in this analysis. However, we were interested in estimating spawner abundance for the entire Green River Chinook spawning habitat (excluding Soos Creek). System-wide total abundance estimates, binomial or hypergeometric model, were generated by expanding tGMR estimates based on the proportion of Chinook redds found downstream of the smolt trap over the course of the entire spawning season. We estimated the proportion of redds upstream of the trap (*ReddUpRate*) as:

$$ReddUpCount \sim \text{Bin}(ReddTotCount, ReddUpRate) \quad (14)$$

where *ReddUpCount* is the redd count upstream of the trap and *ReddTotCount* is the total redd count, all surveys combined. The spawning escapement for the entire Green River (*BinSysWN* or *HypSysWN*) was calculated as:

$$BinSysWN \text{ or } HypSysWN = \frac{BinN \text{ or } HypN}{ReddUpRate} \quad (15)$$

Uninformative Jefferies distributions were used as priors for all proportions (~Beta(0.5,0.5)). For *BinN*, we used an uninformative uniform distribution defined as Uniform(*marks* 10,000). Both adjustments to the GMR spawner abundance estimate were performed in the software R (R Development Core Team 2010) and WinBUGS (Lunn et al. 2000). Uncertainty of the hypergeometric estimate was estimated using WinBUGS based on its probability distribution function. The posterior distribution of the total Green River Chinook spawner abundance was MCMC sampled with 10,000 iterations for burn-in and an additional 90,000 iterations for analysis. Two chains were run with slightly different initial values. Bayesian methods may be affected by lack of convergence of the MCMC sampling and by the modeled priors. We checked the convergence of the models and the sensitivity of the results to the priors with the CODA software (Plummer et al. 2006). We tested the sensitivity of the beta priors (Beta[1,1], Beta[0.01,0.01], and Beta[0.001,0.001]) which have been recommended as vague or uninformative priors for mark-recapture estimates (Rivot and Prévost 2002). We also tested the effect of truncating the prior for the abundance distribution. The script used to run the WinBUGS models can be found in the Appendix.

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 with 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

We used a generalized linear model (GLM) to investigate the effects of carcass location (Newaukum Creek vs. Green River), origin, sex, age, and body length on individual reproductive success. The model was fit using a zero-inflated negative binomial regression in R. A Vuong test was performed to evaluate whether or not the zero-inflated model was an improvement over the standard negative binomial regression model. If no effect of origin, sex or age on reproductive success was detected, abundance of naturally-spawning Chinook by origin, sex, and age was estimated by using the proportions of each origin, sex, and age of the successfully genotyped adult carcass samples.

Evaluate the feasibility and efficacy of a factor for adjusting redd count-based escapement estimates

Evaluations of the feasibility of a redd expansion calibration factor was not possible with just two years (2010 and 2011) of tGMR spawner abundance estimates. This objective will be addressed in a future report.

Relative reproductive success of natural- and hatchery-origin spawners

We used the results of the zero-inflated negative binomial GLM analysis to compare reproductive success of hatchery and wild origin spawners. Whether or not a female salmon spawned can be subjectively determined when sampled as a carcass by the presence of eggs in the abdomen. During carcass surveys, many fish were identified as pre-spawning mortalities. Of the female carcasses, almost 40% were identified as pre-spawning mortalities (Seamons et al. 2012). However, almost half (45%) of the HOR carcasses were fish that had returned to Soos Creek Hatchery and had spent some unknown amount of time there before being released back into the Green River upstream of the smolt trap (referred to hereafter as "re-released HOR"). Eighty-six of 219 (39.2%) female carcasses sampled in the fall of 2011 were judged to be at least 50% unspawned (hereafter "unspawned females"), and almost all (82 of 86) of unspawned females were of hatchery origin. Because these fish were subjected to very different environmental conditions prior to spawning, which may have affected their reproductive success, all carcasses known to be re-released HOR fish, male and female, were dropped from GLM analysis of relative reproductive success.

Results

Sampling

In the fall of 2011, 408 adult Chinook carcasses were sampled in the Green River and Newaukum Creek. Of those, 382 were found upstream of the smolt trap and were of suitable quality for genotyping and tGMR analysis. Overall, the sex ratio of carcasses was female biased with roughly 60% of the genotyped carcasses being female (Table 2). A larger fraction of carcasses sampled in Newaukum Creek than in the Green River were male, but the difference was not statistically significant (Green River – 41%, Newaukum Creek – 45%, Fisher's Exact Test, $P = 0.53$). Overall, more HOR than NOR carcasses were sampled in the Green River basin (Table 2). Within the Green River basin, the fraction of sampled and genotyped HOR carcasses was higher in Newaukum Creek than in the Green River (Fisher's Exact Test, $P = 0.0271$).

Table 2. Summary of adult Green River Chinook carcasses found upstream of the smolt trap site by origin and sex.

	Origin		Sex		Stream
Stream	HOR	NOR	Male	Female	Total
Green River	139	84	92	131	223
Newaukum Creek	117	42	71	88	159
Total	256	126	163	219	382

The Green River smolt trap was operated from January 24 to July 12, 2012 for a total of 3,551 of 4,087 possible hours (87% of the time). Trapping was suspended 29 times due to high water, large releases of hatchery fish, and high recreational use during periods of low catches; the duration of outages ranged from 0.75 to 96.0 hours. Estimates of outmigration were made for trap outage periods (Topping and Zimmerman 2013). A total of 2,548 Chinook (2.8% of the total estimated outmigration) were captured (Table 3). Seasonal trap efficiency was estimated to be ~4.0% (Topping and Zimmerman 2013). The sub-yearling Chinook catch was expanded to an estimated 90,265 ± 21,810 (95% C.I.) outmigrating past the trap during the trapping season. The total basin-wide production estimate for subyearling Chinook was 146,909 sub-yearlings.

Table 3. Weekly Green River juvenile Chinook (WDFW Code 12AS) 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
Pre-	trapping		3,759			
5	23-Jan	29-Jan	1,566	21 (1.3)	16	1.02
6	30-Jan	5-Feb	4,385	72 (1.6)	42	0.96
7	6-Feb	12-Feb	1,012	22 (2.2)	12	1.19
8	13-Feb	19-Feb	2,168	48 (2.2)	22	1.01
9	20-Feb	26-Feb	5,904	48 (0.8)	47	0.80
10	27-Feb	4-Mar	4,772	98 (2.1)	48	1.01
11	5-Mar	11-Mar	3,059	96 (3.1)	36	1.18

Statistical Week (2012)	Start Date	End Date	Estimated Total N	Sampled N (%)	Genotyped N	% of Weekly Outmigration Successfully Genotyped
12	12-Mar	18-Mar	4,193	90 (2.1)	49	1.17
13	19-Mar	25-Mar	2,746	60 (2.2)	32	1.17
14	26-Mar	1-Apr	4,097	85 (2.1)	46	1.12
15	2-Apr	8-Apr	1,808	38 (2.1)	23	1.27
16	9-Apr	15-Apr	1,758	41 (2.3)	23	1.31
17	16-Apr	22-Apr	4,001	74 (1.8)	68	1.70
18	23-Apr	29-Apr	1,807	38 (2.1)	35	1.94
19	30-Apr	6-May	6,337	217 (3.4)	138	2.18
20	7-May	13-May	2,759	105 (3.8)	74	2.68
21	14-May	20-May	3,706	158 (4.3)	109	2.94
22	21-May	27-May	5,033	216 (4.3)	159	3.16
23	28-May	3-Jun	5,194	224 (4.3)	177	3.41
24	4-Jun	10-Jun	7,618	313 (4.1)	298	3.91
25	11-Jun	17-Jun	4,781	204 (4.3)	185	3.87
26	18-Jun	24-Jun	3,866	158 (4.1)	153	3.96
27	25-Jun	1-Jul	1,488	63 (4.2)	52	3.49
28	2-Jul	8-Jul	1,212	44 (3.6)	44	3.63
29	9-Jul	12-Jul	366	15 (4.1)	14	3.83
Post-	trapping		870			
Total			90,265[a]	2,548 (3.4)	1902	

[a]Estimated numbers from Appendix B in Topping and Zimmerman (2013). Topping and Zimmerman (2013) reported 90,260, however numbers in Appendix B add to 90,265.

Genetics, parentage, and sibship analysis

We were able to obtain usable genotypes (≥ 10 loci genotyped) from 194 of 223 carcass samples from the Green River and from 134 of 159 carcass samples from Newaukum Creek for a total of 328 'marked' carcasses for tGMR analysis. Genotyping failed or was insufficient for the remaining samples. There was no statistically significant difference in the sex ratio or origin composition of attempted and successfully genotyped carcasses in either the Green River or Newaukum Creek collections (Fisher's Exact Test, $P > 0.50$ all tests). Genetic diversity was high with average expected heterozygosity = 0.82, providing sufficient power for parentage analysis. No statistically significant deviations from HWE were found at any locus (Table 4).

We were able to obtain usable genotypes (≥ 10 loci genotyped) from 1,902 of 1,996 juvenile tissue samples from the Green River. Twenty-five pairs of matching genotypes were found; one or both individuals of a matching pair were removed from analysis. Genotyping failed or was insufficient for the remaining samples.

Table 4. Genetic parameters for adult carcass samples collected in the fall of 2011 in the Green River and Newaukum Creek upstream of the smolt trap.

Locus	n	N_A	H_e	H_o	F_{IS}	*P* value
Ogo-2	299	16	0.619	0.635	-0.025	0.388
Ogo-4	327	13	0.792	0.807	-0.018	0.590
Oki-100	248	27	0.939	0.931	0.010	0.069
Omm-1080	314	39	0.957	0.962	-0.003	0.888
Ots-201b	323	35	0.945	0.941	0.006	0.086
Ots-208b	323	28	0.936	0.935	0.003	0.629
Ots-211	319	22	0.929	0.903	0.029	0.190
Ots-212	325	24	0.857	0.889	-0.036	0.240
Ots-213	305	28	0.933	0.941	-0.007	0.628
Ots-3M	311	8	0.711	0.678	0.047	0.382
Ots-9	287	5	0.372	0.362	0.028	0.827
Ots-G474	283	11	0.685	0.682	0.006	0.187
Ssa-197	323	29	0.936	0.932	0.006	0.146
Ssa-408	320	17	0.863	0.875	-0.012	0.735
Average	307.6	21.6	0.820	0.820		

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

Maximum likelihood parentage assignment using FRANz identified 383 parentage assignments to 119 individual genotyped carcasses (36.3% of genotyped carcasses). Twenty-seven assigned parent-offspring pairs were rejected because the offspring or the parent had less than 14 loci genotyped and the parent assignment was made with one mismatch. The CV of the preliminary hypergeometric abundance estimates based on the three short length COLONY2 runs exceeded the cutoff of 5%, so a medium length COLONY2 run was performed with all other parameters the same as the short runs. The medium length run of COLONY2 identified 396 parentage assignments to 112 individual genotyped carcasses (34.1% of genotyped carcasses). The number of unique, unsampled parents, inferred through COLONY2 sibship analysis was 857.

Parentage assignments made with FRANz and the medium length run of COLONY2 were identical for 3,741 out of 3,804 (98.3%) possible parentage assignments. Of the 63 different parentage assignments, 15 were of the 27 FRANz parentage assignments that were rejected due to missing data and mismatches between parent and offspring. In four cases, FRANz assigned a single parent and COLONY2 assigned two parents, one of which was the same parent assigned by FRANz. In 34 cases, COLONY2 assigned a single parent and no parent was assigned by FRANz. In the remaining 25 cases, a single parent was assigned by FRANz and no parent was assigned by COLONY2.

The number of offspring assigned to sampled carcass parents that had at least one offspring assigned ranged from 1 to 18 (mean = 3.54), and the number of offspring assigned to inferred, unsampled parents ranged from 1 to 28 (mean = 3.98). The distribution of the number of offspring assigned to sampled carcass parents assigned at least one offspring was significantly different from that assigned to inferred, unsampled parents (P = 0.0026, Figure 2).

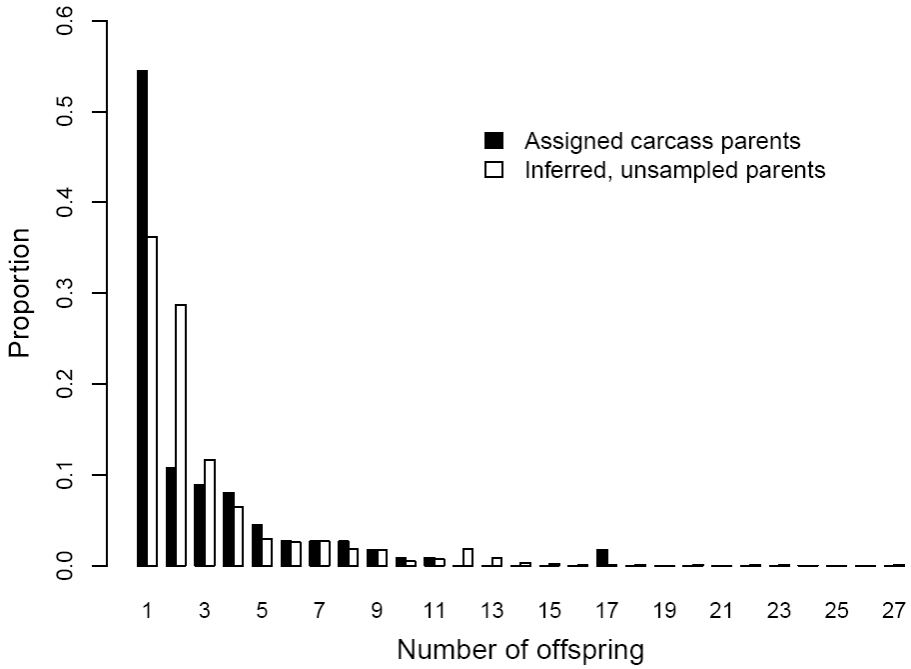

Figure 2. Distribution of the number of offspring assigned to either sampled carcasses or inferred, unsampled parents using the sibship and parentage analysis algorithms in COLONY2. The distributions were significantly different, based on a permutation test (P = 0.0026), suggesting that either our carcass sample was not representative of the population or that the algorithms of COLONY2 incorrectly lumped unrelated individuals into families at the half- or full-sibling level or split half- or full-sibling families into smaller families.

tGMR spawner abundance estimates

From 23 April, 2012, to 12 July, 2012, 730 adipose fin-marked hatchery-produced juveniles were captured in the Green River smolt trap. The adipose fin clip marking rate was high for Soos Creek Hatchery releases (98.2%) with slightly lower rates for Soos Creek Hatchery CWT and Palmer Rearing Ponds (91.4% and 92.3%, respectively). Juveniles with CWT were not counted at the smolt trap, however, based on the proportion of DIT fish released from Soos Creek Hatchery and the number of adipose fin marked juveniles trapped, the expected number of genotyped DIT juveniles was two. The estimated total number of unmarked hatchery-produced juveniles caught in the smolt trap was 33. Given weekly genotyping rates, 24 were estimated to have been genotyped. Thus, Green River capture numbers for the binomial estimator were reduced by 48 (number genotyped times two, see Methods section) and by 24 for the hypergeometric estimator.

A total of 298 redds were counted in the Green River and Newaukum Creek (excluding Soos Creek; A. Bosworth, WDFW, personal communication). Of those, 6 (2.0%) were found downstream of the smolt trap and 292 (98.0%) were found upstream of the smolt trap.

Binomial tGMR estimates of BY 2011 Green River Chinook spawner abundance exceeded 3,000 using parentage data from either FRANz or COLONY2 (Table 5). The hypergeometric tGMR estimate was less than the binomial estimates but was still much higher than that based on redd counts, which was 993. Overall Chinook salmon spawner abundance estimates (binomial and hypergeometric) for the entire Green River were around three times higher than the estimate based on redd counts. Coefficients of variation for all tGMR estimates, based on estimated variance, were below the recommended level of 15% (Table 5).

Table 5. BY 2011 Green River Chinook tGMR population abundance estimates.

	binomial	binomial	hypergeometric	Redd and trap count
Parentage algorithm	FRANz	COLONY2	COLONY2	
MARKS	328	328	328	
CAPTURES	1878.0	1877.7	944.6	
RECAPTURES	383	396	112	
N - upstream of trap	3236	3131	2806	
Var – upstream of trap	53485.7	48849.8	42357.5	
N – system-wide[a]	3308	3201	2868	993
(95%CI)	(2867-3798)	(2778-3671)	(2493-3321)	
Var – system-wide	56711.6	51796.2	44867.4	
CV (%)- system-wide	7.2	7.1	7.4	

[a]-System-wide estimate includes all production downstream of the Green River smolt trap for the Green River Chinook population, which includes the lower Green River mainstem, but excludes natural production in Soos Creek.

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

The effective number of breeders (N_b) for BY 2011 Green River Chinook upstream of the smolt trap, based on the medium length run of COLONY2, was estimated to be 1,315 (BH, 95%CI = 1,306.3 – 1,322.7) or 1,003 (CSHS, 95%CI = 999.1-1006.2; Figure 3). The continuous smooth hockey stick model fit the data better than the Beverton-Holt model (BH average AIC_c = 817.5, 95%CI = 796.3 – 837.9; CSHS average AIC_c score = 747.8, 95%CI = 716.0 – 778.0).

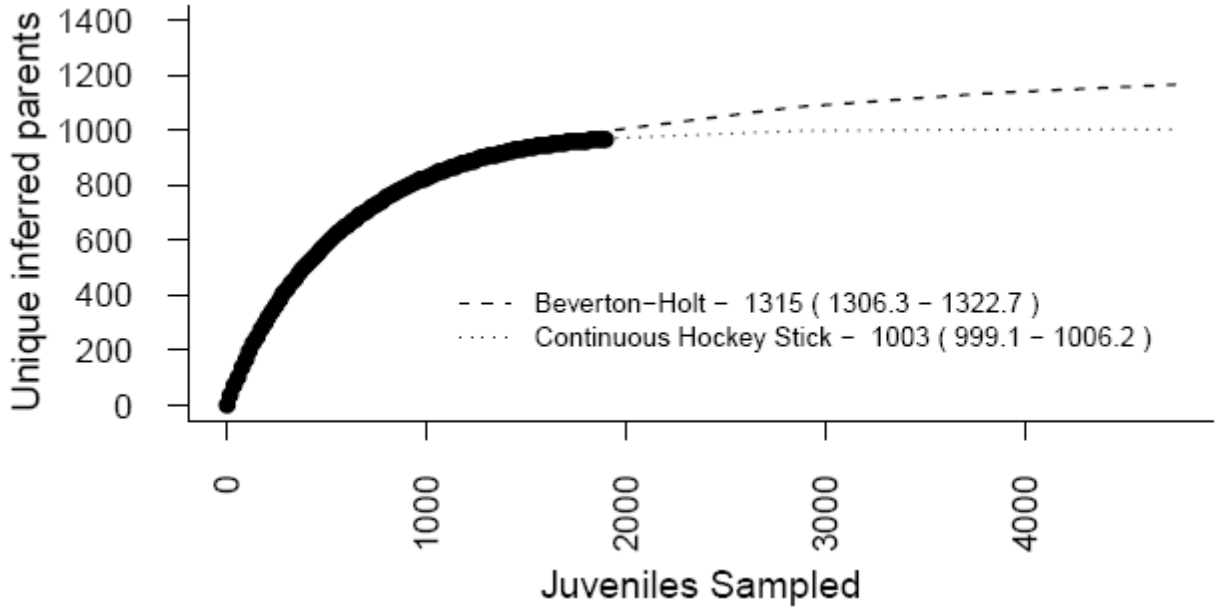

Figure 3. BY 2011 rarefaction curve estimates of the effective number of breeders (N_b) of Green River Chinook, upstream of the Green River smolt trap, based on parentage assigned using COLONY2. Dots represent the means of 10,000 samples of the dataset at each subsample value. Lines represent the values 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. The mean value (and the 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

The zero-inflated negative binomial analysis was an improvement on the standard negative binomial regression model ($P = 0.0073$), so results presented are from the zero-inflated model. Only fork length was significantly related to reproductive success ($P = 0.000473$), so abundance could be partitioned based on the frequencies in the genotyped carcass sample (Table 6). Of the genotyped carcasses, 70% (229) were of hatchery origin and 30% (99) were female (Table 6). The sex ratio of HOR adults was female biased and the sex ratio of NOR adults was nearly equal (Table 6). Most sampled carcasses were age 4 (79% of aged fish). No age 2 females were detected, but four HOR and one NOR age 2 males (jacks) were sampled and genotyped.

Table 6. Summary of successfully genotyped brood year 2011 adult Chinook carcass samples.

		age					
Origin	Sex	2	3	4	5	Undetermined	Total
HOR	Male	4	21	40	0	27	92
	Female	0	9	87	5	36	137
	Total	4	30	127	5	63	229
NOR	Male	1	12	33	0	3	49
	Female	0	2	41	1	6	50
	Total	1	14	74	1	9	99

Evaluate the feasibility and efficacy of a factor for adjusting redd count-based escapement estimates

This is the second year of the project. The feasibility of adjusting redd count-based escapement estimates using tGMR estimates will be evaluated after an additional year is completed.

Relative reproductive success of natural- and hatchery-origin spawners

Before removing re-released adults, the average reproductive success of NOR Chinook was nearly three times that of HOR Chinook (2.14 offspring per parent vs. 0.80 offspring per parent, respectively). Genotypes were obtained from187 of the 219 females; 69 of 187 were from re-released HOR females, 68 of 187 were from all other HOR females and 50 were from NOR females (Table 7). Seventy-six percent (53 of 69) of the re-released HOR females were identified as unspawned, a much higher frequency than in all other HOR female collections or NOR female collections ($X^2_{0.05,2} = 70.179$, $P << 0.0001$; Table 7). In addition, as expected, unspawned re-released females had a much lower average reproductive success (Table 7).

Table 7. Sample size and average reproductive success (RS) of female hatchery origin recruit (HOR) or natural origin recruit (NOR) Green River Chinook sampled as carcasses and identified as either those that died before spawning more than half of their eggs ("Unspawned") or those that did not die before spawning ("Spawned").

	Re-released HOR		All other HOR		NOR	
	n[b]	RS	n	RS	n	RS
Unspawned	53	0.40	15	0.53	4	2.00
Spawned	16	0.13	53	1.21	46	2.41
Total	69		68		50	

[a]RS is the untransformed number of juvenile offspring per female per group, identified through genetic parentage assignment.
[b]n is the sample size of genotyped individuals in each group.

After removing re-released fish, the average reproductive success of NOR Chinook was still higher than that of HOR fish (2.16 offspring per parent vs. 1.17 offspring per parent). Reproductive success was not related to location (Newaukum vs. Green River, $P = 0.1562$), sex ($P = 0.1920$), age ($P = 0.9297$), or origin ($P = 0.5138$), but longer fish produced more offspring ($P = 0.0003$). While there was no significant effect of origin, the zero-inflated model indicated that hatchery fish were more likely to have a zero value of reproductive success ($P = 0.0027$).

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 2011 Green River tGMR abundance estimates, like the 2010 Green River tGMR abundance estimates (Seamons et al. 2012) and the 2011 Snohomish River tGMR abundance estimates (Seamons et al. 2013), were several times larger than the redd based estimates for the system and for the area upstream of the smolt traps (Table 5, **Error! Reference source not found.**). 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) regardless of whether or not they construct a redd, 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 eggs within their abdomen, are judged to be unspawned are found on the spawning grounds during spawning surveys every year; in 2011, ~40% of female carcasses were identified as pre-spawning mortalities. In 2011, 1,689 live adult Chinook were taken from Soos Creek Hatchery, floy tagged, and released upstream of the smolt trap in the Green River by the Muckleshoot Tribe. Carcasses of these fish were subsequently sampled on the spawning grounds ("re-released" fish). Nearly 77% of these re-released females were identified as pre-spawning mortalities. The tGMR method estimated these fish in the final abundance estimate since they were sampled and some of them produced offspring, but if they failed to dig a redd, they were not counted in any redd count-based estimate. This was obviously not true since a) the fraction of fish called unspawned contained some fish that were partially spawned, and b) some parentage assignments were made to fish called unspawned. However, assuming 40% was a maximum fraction of unspawned females and that

unspawned females did not dig redds, adjusting the redd count-based estimate by the unspawned fraction produced a redd count-based estimate much closer to the tGMR estimate (2,482).

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 5). Most parentage assignments were the same. The few that were different involved equivocal assignments based on incomplete genetic data plus a genetic mismatch between parent and offspring and 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 lower, but not significantly different, than those inferred by COLONY2. The resulting differences in spawner abundance estimates were relatively small (within ~3%), 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. The distribution of reproductive success of sampled Chinook parents was different than that of the inferred, unsampled parents in 2011 (Figure 2; but was not significantly different in 2010 [Seamons et al. unpublished data]), suggesting either the carcass samples were unrepresentative of the population, or COLONY2 incorrectly lumped individuals into families. Incorrect lumping would produce a downwardly biased abundance estimate. On the other hand, assuming that the COLONY2 output was correct, the inference is that unsuccessful adults were sampled at too high of a rate, which would produce an upwardly biased abundance estimate. The 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 weeks 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. Using either algorithm, 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 Green River system, the two types of immigrants were 1) untagged (no CWT) and unmarked (no adipose fin clip) hatchery origin recruit (HOR) juvenile Chinook presumed to have been intentionally or accidentally released from Palmer Rearing Ponds, Keta Creek Hatchery, and Soos Creek Hatchery; and 2) natural origin recruit (NOR) juvenile Chinook produced in Big Soos Creek. If left uncorrected, the presence of these juvenile immigrants in our sample would inflate capture numbers and upwardly bias estimates of *N*. In our study, some 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 facilities. However, juveniles were unclipped when transferred from Soos Creek to Keta Creek Hatchery. Any leakage from Keta Creek Hatchery prior to adipose fin clipping was not factored into calculations. In addition, short of sampling every adult Chinook spawning in Soos Creek for parentage analysis, there is no way to identify all Soos Creek NOR juvenile immigrants, so there was no correction for any Big Soos Creek NOR juvenile immigrants. Natural production of juvenile Chinook is thought to be substantial (Heller 2012), so the potential for 'immigration' under the tGMR methods appears high. Given that HOR juveniles released from Soos Creek Hatchery are caught in the smolt trap, it is likely that some Soos Creek NOR juveniles also end up caught in the smolt trap. Presence of these immigrants would bias the tGMR abundance estimate high.

Correcting capture numbers for total estimated number of immigrants 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 not created using hatchery spawning protocols (i.e. one female is spawned with one male, Soos Creek Fall Chinook HGMP; http://wdfw.wa.gov/hatcheries/hgmp/pdf/puget_sound/soos_cr_chin_hgmp_final_draft_040313.pdf), so if full-siblings were among the genotyped HOR juveniles, corrected capture numbers and the hypergeometric estimates may be biased slightly low.

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 outmigrate 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 (if we used them) would also be considered 'emigrants' since their offspring would rarely or never be caught in the smolt trap. However, this type of "emigration" only changes the existing heterogeneity in capture probabilities at the second sampling event, which is the subject of assumption 5. In theory, because of this characteristic of the tGMR methodology, some fraction of the downstream spawners could be included in our tGMR without biasing the estimate, eliminating the need to expand based on redd counts. This idea needs to be tested by simulation before including in the normal protocol.

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. Rawding et al.(2013) addressed this concern by evaluating the relationship of reproductive success (i.e., capture probability in the second event) and various characteristics of the carcasses known to be correlated with capture probabilities in the first event (e.g., age, body length). A non-significant result was interpreted as independence. Green River carcass collections were likely biased with regard to age and body size (Murdoch et al. 2010; Zhou 2002), and these traits were positively correlated with Chinook reproductive success in Green River Chinook as has been shown in other systems (Williamson et al. 2010). Thus, it is possible the Green 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.

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). Capture probabilities of both events may be correlated when too many fish are in the carcass collection that "emigrated" or failed to spawn or produce offspring (e.g., prespawning mortalities, Keefer et al. 2010). This 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. Carcasses are much easier to collect in Newaukum Creek than in the Green River due to the lower volume of water. Indeed, 40% of the sampled and genotyped carcass collections were from Newaukum Creek, but only 15% of Green River system redds were counted in Newaukum Creek. However, there was no statistically significant difference in the reproductive success of fish from either location suggesting that the 2011 tGMR abundance estimate was unbiased in this regard.

Independence of sampling events may be violated due to habitat associations of carcass recovery and individual reproductive success. For example, spawning habitats associated with greater stream complexity may lead to increased survival to the fry stage and to reduced carcass recoveries. Similarly, fish swimming or carcasses drifting out of the sampling area upstream of the smolt trap could lead to non-independence of sampling events if the drifting carcasses were those fish whose reproductive success was much higher or much lower than other fishes or those whose offspring were more likely to be captured in the smolt trap. This is likely to affect females more than males due to their spawning behaviors (Healey 1991). Female Chinook salmon guard their spawning site until death and male Chinook cover the spawning grounds looking for mates. Consequently, female carcasses tend to be recovered a relatively short distance downstream of their spawning site (100s of meters), while male carcasses are found much further away (1000s of meters) (Murdoch et al. 2009). Reach specific habitat data were not recorded during carcass collection in 2011, so a direct test of the effects of habitat complexity on reproductive success could not be performed. However, the male-only binomial tGMR abundance estimate should be unbiased in this regard. The male tGMR abundance estimate was N=1691, by itself still nearly twice the redd count-based estimate. In addition, there is evidence that parental effects, independent of habitat, play a significant role in determining reproductive success (Johnson et al. 2012). Thus, while it could not be tested, it seems unlikely that the tGMR estimates are significantly biased due to habitat associations or carcass drift.

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

Estimates of N_b were smaller than tGMR spawning escapement estimates, suggesting that roughly 67% of the spawners failed to produce offspring. N_b estimates were much closer to the redd count-based estimates. 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 the Stillaguamish River 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 was within the expected range.

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 figure 3 appeared to be reaching an asymptote. The estimated numbers of unique sampled and inferred parents (unadjusted hypergeometric capture number, 944) was not very different from the estimated asymptotic values estimated through rarefaction (1,003), 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 Big Soos Creek NOR juvenile immigrants and hatchery-produced immigrants are of concern. Identification of immigrant 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 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 non-immigrant NOR population. If few families exist, the bias due to immigrants will be large, but if the number of families is the same or more than those in the immigrant population, the bias will be very small or non-existent. An estimated 24 HOR immigrants were genotyped, around 5% 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. The smolt trap was in place throughout most of the emigration period. Reproductive success data from BY 2010 and 2011 suggest that, while there were 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, the Green River N_b estimate was 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. The smolt trap collection was 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., the canyon reach) and all juveniles produced in spawning reaches upstream of the smolt trap must pass by or through the smolt trap. However, the juvenile sample also likely contained unaccounted for immigrants.

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 existed, but within family variance was large. The parentage assignment results showed that multiple members of the same family were often sampled, but whether or not this differs from random expectations is unknown since the underlying distribution of reproductive success is also unknown.

tRC Additional concerns – The tRC estimate of N_b was 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 trap was distributed the same as that of spawners upstream of the trap. 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.

Relative reproductive success and partitioning abundance

Studies of hatchery and wild Chinook reproductive success have found hatchery origin adults to have lower reproductive success than natural origin adults, particularly the males (Williamson et al. 2010). In Green River Chinook, the mean reproductive success of HOR Chinook was lower than that of NOR Chinook in 2011, but the difference was not statistically significant. However, HOR fish were more likely to have zero detected offspring than NOR Chinook.

Fewer offspring were detected from female Chinook identified as pre-spawning mortalities in 2011. Most of these females were fish that had returned to Soos Creek Hatchery, had been held for some time, before being tagged and released back into the Green River upstream of Big Soos Creek (re-released fish). This was the second year of that project and protocols were still being developed, so pre-spawning mortalities this high should not occur in the future. Indeed, in 2012, the number of pre-spawning mortalities overall had dropped as had the fraction of re-released fish identified as pre-spawning mortalities (data not shown).

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 by WDFW biologists. Genotyping of adult carcass tissues and juvenile tissues was performed and completed according to schedule. We improved code for both the tGMR and tRC analysis, correcting the way numbers of unmarked and untagged hatchery immigrants are estimated and improving the precision of the estimate of N_b.

The tGMR method relied on at least one untestable assumption, the assumption that the sampling events were independent and the first sampling event was representative. In addition, some unmarked and untagged hatchery origin juveniles may also have been unaccounted for due to the difference in QA/QC rates between Soos Creek Hatchery and Palmer Ponds and the uncertainty of from which hatchery source a particular fish was released. Starting with releases in 2014, juvenile Chinook released from Palmer Ponds will not be externally marked and they will be released from Palmer Ponds during the trapping period instead of after trapping is done. In order to obtain an unbiased tGMR estimate for BY 2013 and future years, all hatchery broodstock will have to be genotyped, which would allow for complete identification and enumeration of all unmarked and untagged hatchery fish.

The assumption of a closed population was likely not met due to "immigration" of natural origin juveniles produced in Big Soos Creek. Immigration from natural origin Big Soos Creek fish could be estimated if total production were known and if some large fraction of juvenile outmigrants could be uniquely marked. Alternatively, natural production in Big Soos Creek could be added to the Green River escapement estimates. Carcasses from Big Soos Creek could be genotyped and used as marks and some fraction of outmigration juveniles from Big Soos Creek could be intentionally sampled.

While we can be certain that some assumptions were met, some were undoubtedly not met. Multiple sources of bias of small effect may have a large effect when combined. The cumulative effects of small violations of multiple assumptions with regard to Green River Chinook will be evaluated separately by analysis of simulated data in future documents.

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Appendix

R script used to run WinBUGS for the binomial estimator:

```
library(R2WinBUGS)
library(BRugs)
library(modeest)

BRugsmodel <- function() ############ start of model ######################
{
 #estimate QC rates and uncertainty
 PADpresent~dbin(PADrate,PADsample)
 SADpresent~dbin(SADrate,SADsample)

#Jefferies priors for the QC tag/mark rates
 PADrate~dbeta(0.5,0.5)
 SADrate~dbeta(0.5,0.5)

 #Estimate number of unclipped hatchery produced fish to subtract from Captures
 for (i in 1:4){
  adjADcount[i]<- ADcount[i]/PADrate
  lostADcount[i]<- adjADcount[i]-ADcount[i]
  GenoADcount[i]<-lostADcount[i]*GenoRate[i]
 }

 #Soos - Estimate true number of unclipped AD to subtract from Captures
 for (i in 5:12){
  adjADcount[i]<- ADcount[i]/SADrate
  lostADcount[i]<- adjADcount[i]-ADcount[i]
  GenoADcount[i]<- lostADcount[i]*GenoRate[i]
 }

 #Total number of sampled cheaters (unclipped hatchery produced fish)
 TotCheaters <- sum(lostADcount[])#+sum(lostCWTcount[])

 #Adjusted total number of genotyped natural origin juveniles
 TotadjJuveCount <- Gcount + sum(GUcount[])-TotGCheaters

 #Total number of genotyped cheaters
 TotGCheaters <- sum(GenoADcount[])#+sum(GenoCWTcount[])

 #Adjusted binomial captures
 adjBinCaptures <- TotadjJuveCount*2

 #priors for proportion marked and abundance Binomial
 markRate~dbeta(0.5,0.5)
 BinN~dunif(marks,10000)

 #likelihood for mark-recapture adjusted for unmarked hatchery fish
 marks ~dbin(markRate,BinN)
 BinRecap ~dbin(markRate,adjBinCaptures)
```

```
  #calculate % redds above trap & estimate total abundance adjusted for cheaters
  ReddUpCount ~dbin(ReddUpRate,ReddTotCount)
  ReddUpRate ~dbeta(0.5,0.5) # prior for % redd above trap
  BinSysWN <- BinN / ReddUpRate # Binomial systemwid total abundance adjusted for cheaters
 }

 ###### end of model ###################

 # Save BUGS description of the model to working directory
 model.file <- file.path("C:\\FRANz_Binomial_model.txt")
 write.model(BRugsmodel, model.file)


 # Package all the stuff to be handed over to WinBUGS
 # Bundle data
 win.data <- list(ADcount=c(39,204,41,12,23,23,37,137,105,83,23,3),
          #CWTcount=c(0,0,0,0,2,2,2,9,7,5,2,2),
          PADpresent=1846,
          PADsample=2000,
          SADpresent=982,
          SADsample=1000,
          #CWTpresent=457,
          #CWTsample=500,
          Gcount=464,
          ReddUpCount=292,
          ReddTotCount=298,
          marks=328,
          BinRecap=383,
          GenoRate=c(0.9210,0.6359,0.7047,0.6898,0.7361,0.7901,
                    0.9520,0.9068,0.9683,0.8253,1.0000,0.9333),
          GUcount=c(35,138,74,109,159,177,298,185,153,52,44,14))

 # Function to generate starting values
 inits <- list(list(ReddUpRate=0.9,BinN=4000),
         list(ReddUpRate=0.8,BinN=3500))

 # Parameters to be monitored (= to estimate)
 params <-
c("TotCheaters","TotGCheaters","TotadjJuveCount","BinN","BinSysWN","markRate")

 # MCMC settings
 nc <- 2                          # Number of chains
 ni <- 100000                     # Number of draws from posterior (for each chain)
 nb <- 10000                           # Number of draws to discard as burn-in
 nt <- 1                          # Thinning rate

 # Start Gibbs sampler: Run model in WinBUGS and save results in object called out
 out <- bugs(
  data = win.data,
  inits = inits,
```

```
  parameters.to.save = params,
  model.file = "FRANz_Binomial_model.txt",
  bugs.directory="C:/data/Winbugs/WinBUGS14",
  program=c("WinBUGS"),
  n.thin = nt,
  n.chains = nc,
  n.burnin = nb,
  n.iter = ni,
  bugs.seed=666,
  debug = TRUE,
  DIC = TRUE,
  working.directory = getwd())
```

R script used to run WinBUGS for the hypergeometric estimator:

```
library(R2WinBUGS)          # Load the R2WinBUGS library
library(BRugs)
library(modeest)


BRugsmodel <- function() ############ start of model ######################
{
 #estimate QC rates and uncertainty
 PADpresent~ dbin(PADrate,PADsample)
 #CWTpresent~ dbin(CWTrate,CWTsample)
 SADpresent~ dbin(SADrate,SADsample)

 #Jefferies priors for the QC tag/mark rates
 PADrate~ dbeta(0.5,0.5)
 SADrate~ dbeta(0.5,0.5)

 #Estimate number of unclipped hatchery produced fish to subtract from Captures
 for (i in 1:4){
  adjADcount[i]<- ADcount[i]/PADrate
  lostADcount[i]<- adjADcount[i]-ADcount[i]
  GenoADcount[i]<- lostADcount[i]*GenoRate[i]
 }

 #Soos - Estimate true number of unclipped AD to subtract from Captures
 for (i in 5:12){
  adjADcount[i]<- ADcount[i]/SADrate
  lostADcount[i]<- adjADcount[i]-ADcount[i]
  GenoADcount[i]<- lostADcount[i]*GenoRate[i]
 }

 #Total number of genotyped cheaters
 TotGCheaters<- sum(GenoADcount[])#+sum(GenoCWTcount[])

 #Total number of genotyped natural origin juveniles unadjusted for cheaters
 TotGJuveCount<- Gcount + sum(GUcount[])
```

```
  #Adjusted hypergeometric captures assumes all from different families
  HypCaptures[2]<- HypCaptures[1] - TotGCheaters

#there is no function in WinBUGS for hypergeometric distribution
#likelihood with zero trick for hypergeometric and temp to constrain logfact to positive

for(i in 1:2){
                min[i]<- HypCaptures[i]+HypMarks[i]-HypRecap[i]
                HypN[i]~ dunif(min[i],max[i])
                zeros[i]<- 0
                zeros[i]~ dpois(lglkHypN[i])  # likelihood is exp(-lglk)
                tmp1[i]<- max(0,HypMarks[i]-HypRecap[i])
                tmp2[i]<- max(0,HypCaptures[i]-HypRecap[i])
                tmp3[i]<- max(0,HypN[i]-HypCaptures[i])
                tmp4[i]<- max(0,HypN[i]-HypMarks[i])
                tmp5[i]<- max(0,tmp4[i]-tmp2[i])
                lglkHypN[i]<- -((logfact(HypMarks[i]) - logfact(HypRecap[i]) -
logfact(tmp1[i])) + (logfact(tmp4[i]) - logfact(tmp2[i]) - logfact(tmp5[i])) - (logfact(HypN[i]) -
logfact(HypCaptures[i]) - logfact(tmp3[i])))+10000     # -log(likelihood)
        }

#calculate estimate total abundance based on % redds above smolt trap
  #calculate % redds above trap & estimate total abundance adjusted for cheaters
  ReddUpCount ~dbin(ReddUpRate,ReddTotCount)
  ReddUpRate ~dbeta(0.5,0.5) # prior for % redd above trap
  HypSysWN[1]<- HypN[1]/ReddUpRate # unadjusted for cheaters
  HypSysWN[2]<- HypN[2]/ReddUpRate          # adjusted for cheaters

}###### end of model ###################

# Save BUGS description of the model to working directory
model.file <- file.path("C:\\ Simple_Hypergeometric_model.txt")
write.model(BRugsmodel, model.file)


# Package all the stuff to be handed over to WinBUGS
# Bundle data
win.data <- list(ADcount=c(39,204,41,12,23,23,37,137,105,83,23,3),
        #CWTcount=c(0,0,0,0,2,2,2,9,7,5,2,2),
        PADpresent=1846,
        PADsample=2000,
        SADpresent=982,
        SADsample=1000,
        #CWTpresent=457,
        #CWTsample=500,
        Gcount=464,
        ReddUpCount=292,
        ReddTotCount=298,
        HypMarks=c(328,328),
        HypRecap=c(112,112),
        HypCaptures=c(969,NA),
```

```
                max=c(10000,10000),

GenoRate=c(0.9210,0.6359,0.7047,0.6898,0.7361,0.7901,0.9520,0.9068,0.9683,0.8253,1.0000,0.9333),
                GUcount=c(35,138,74,109,159,177,298,185,153,52,44,14))

        # Function to generate starting values
        #inits <-
list(list(ReddUpRate=0.9,SADrate=0.99,PADrate=0.95,CWTrate=0.95,HypN=c(4000,4000) ),
        #
list(ReddUpRate=0.8,SADrate=0.95,PADrate=0.99,CWTrate=0.99,HypN=c(5000,5000) ))
        inits <- list(list(ReddUpRate=0.9,SADrate=0.99,PADrate=0.95,HypN=c(4000,4000) ),
                list(ReddUpRate=0.8,SADrate=0.95,PADrate=0.99,HypN=c(5000,5000) ))

        # Parameters to be monitored (= to estimate)
        #params <-
c("TotGCheaters","HypN[1]","HypN[2]","HypSysWN[1]","HypSysWN[2]","HypCaptures[2]","CWTrat
e","SADrate","PADrate")
        params <-
c("TotGCheaters","HypN[1]","HypN[2]","HypSysWN[1]","HypSysWN[2]","HypCaptures[2]","SADrate
","PADrate")

        # MCMC settings
        nc <- 2                         # Number of chains
        ni <- 100000                    # Number of draws from posterior (for each chain)
        nb <- 10000                             # Number of draws to discard as burn-in
        nt <- 1                         # Thinning rate

        # Start Gibbs sampler: Run model in WinBUGS and save results in object called out
        out <- bugs(
        data = win.data,
        inits = inits,
        parameters.to.save = params,
        model.file = "Simple_Hypergeometric_model.txt",
        bugs.directory="C:/data/Winbugs/WinBUGS14",
        program=c("WinBUGS"),
        n.thin = nt,
        n.chains = nc,
        n.burnin = nb,
        n.iter = ni,
        bugs.seed=666,
        debug = TRUE,
        DIC = TRUE,
        working.directory = getwd())
```