2013 Progress Report: Chinook salmon abundance in the Stillaguamish River estimated using genetic mark-recapture analyses

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Executive Summary

The Stillaguamish River Chinook salmon are one of seven escapement indicator stocks in Puget Sound designated by the Chinook salmon Technical Committee (CTC) of the Pacific Salmon Commission (PSC). The escapement indicator stocks reflect effectiveness of 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 Stillaguamish River Chinook salmon are a stock of concern due to declines from historic levels, current low abundance and resultant limitations this imposes on fisheries management. In addition, this stock was identified as a sentinel stock in the latest Pacific Salmon Treaty. Estimates for historic Chinook salmon returns ranged from 9,700-13,321 per year as compared to an average of 1080 in more recent years (1996-2003). Although their overall harvest rate is the lowest of all CTC indicator stocks, in large part due to lack of abundance, from 1999-2006 the mean Canadian exploitation rate (ER) for this stock was ~15%, which was nearly double the exploitation rate in Southern United States (SUS) fisheries (8.1%).

The Sentinel Stocks Program funded this study to estimate the Chinook salmon spawning escapement using a genetic mark-recapture (GMR) protocol. The tGMR protocol employs genotypes from carcasses collected in the fall and outmigrants captured via smolt trapping during the following winter and spring. We assigned smolts collected in the mainstem in 2013 to their parents: hatchery- and natural or wild-origin natural spawners collected in 2012. Wild-origin spawners were more abundant but there was no significant difference in production between the spawner types. Using a pooled Lincoln-Peterson estimate (genotyped spawners = marks, genotyped outmigrating juveniles = captures, spawners assigning to juveniles = recaptures) we calculated spawner abundance and compared this abundance estimate to tGMR estimates calculated for brood years 2007-2012, and compared each year's tGMR estimates to estimates derived from redd count expansions (Table 3). In all years except 2011 the confidence interval for the tGMR estimate encompassed the redd-based estimate.

Stillaguamish Chinook salmon juveniles presented challenges to the tGMR study design with unmarked hatchery juveniles (juveniles leaving the hatchery upstream of the smolt trap with their adipose fins intact) and yearling juveniles (juveniles leaving the system after 14 months, rather than two months) in the smolt samples. If present and unaccounted for, unmarked hatchery juveniles and yearlings inflate abundance estimates because they increase capture numbers yet have no possible parents in the "Mark" pool. We identified unmarked hatchery juveniles by assigning smolts to the hatchery broodstock and removed them prior to analyses. We identified yearlings by plotting smolt lengths and capture dates and observing outlier smolts that were much longer that average smolt lengths for each time strata, and removed them from analyses. Because there were few unmarked hatchery fish and Chinook salmon yearlings, adjustments resulted in small changes in the tGMR estimates. The tGMR results presented here include corrections for unmarked hatchery juveniles and yearlings.

Despite the opportunistic sampling for some brood years, the coefficient of variation (CV) based on Bailey's binomial model did not meet the CTC standard of 15% in only the first of six years. The Sentinel Stocks program provided $85,021 US for the Stillaguamish River Chinook salmon tGMR project in 2013.

Introduction

In Puget Sound, seven Chinook salmon stocks are used as escapement indicator stocks by the Chinook salmon 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 summer/fall. The escapement indicator stocks monitor the effectiveness of the management regimes and, if necessary, their status may trigger additional management actions in AABM and ISBM fisheries. The U.S. members of the CTC (USCTC) developed data standards for stock-specific assessments of escapement, terminal runs, and abundance forecasts against which existing stock assessment programs could be evaluated (USCTC 1997).

The USCTC (1997) found that individual escapement estimates in Puget Sound range from very good to very poor. The most apparent shortcomings 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 addresses these shortcomings in the Stillaguamish River and provides information on how best to maintain survey efforts meeting USCTC data standards. This project focuses on natural summer/fall Chinook salmon that originate from the Stillaguamish River System, a producer of wild Chinook salmon in Puget Sound, and the only stock identified during the creation of the Sentinel Stocks Program (SSP).

Stillaguamish River Chinook salmon (summer/fall fingerlings), including hatchery supplementation releases, are a stock of concern due to low abundance. Although their overall harvest rate is the lowest of all CTC indicator stocks, in large part due to low abundance, from 1999-2006 the mean Canadian exploitation rate (ER) for this stock was ~15%, which was nearly double the ER in Southern United States (SUS) fisheries (8%). Over this same period the ER for this stock in Alaskan fisheries was 4.5% (M. Alexandersdottir, NWIFC, pers. comm.). Although the distribution of exploitation across fisheries is different (CTC 2008), Skagit River spring Chinook salmon fingerlings are the only other stock with a roughly comparable overall ER.

The North Fork Stillaguamish River supports a summer Chinook salmon population that has been managed since 1980 as an “integrated stock”. The integrated stock is maintained at the Harvey Creek Hatchery, with both hatchery-origin recruits (HOR) and natural-origin recruits (NOR) serving as broodstock (J. Griffith, Stillaguamish Tribal biologist, pers. comm.). The South Fork Stillaguamish River mainstem and several tributaries support a fall Chinook salmon population that transitioned in 2010 to an integrated stock, following the model of the North Fork program. The fall program includes captive brood collected from the South Fork Stillaguamish, along with annual collections of mature fall adults from various locations throughout the watershed.

Objectives

The primary objective of this project is to: 1) estimate the abundance of Chinook salmon spawners (N) and effective breeders (N_b) in the Stillaguamish River upstream of the smolt trap site (RM 6) for brood year 2012 using genetic abundance methods. The secondary objectives of this study are to: 2) estimate the natural spawning Chinook salmon abundance by origin (hatchery or natural), sex and age, and 3) estimate a redd expansion calibration factor from historic redd-based escapement estimates and possible future redd counts. The data collected for this project provide a genetic baseline for these population estimates. In earlier work (Small et

al. 2012) the project employed data collected for prior research objectives including genetic samples from fall spawning periods 2007, 2008, 2009, and 2010. Because these spawner samples were collected prior to the design of the tGMR project, only natural-origin spawners were sampled. Starting with brood year 2011, all spawners in spawning areas were sampled, regardless of origin. Abundance by origin will thus be estimated for brood year 2012 spawners. We propose meeting the bilateral data standards for estimating the number of natural origin spawners including: 1) spawning escapement estimates with an average estimated coefficient of variation (CV) of 15% or less; and 2) these estimates will be consistent and unbiased.

Methods

Study Site

The Stillaguamish River originates in foothills of the Cascade Mountains in the northeastern portion of the Puget Sound watershed, that was formerly densely forested (Figure 1). There are two main tributaries, the North Fork (NF, about 45 miles long) and the South Fork (SF, about 30 miles long), each with numerous smaller tributaries in their basins. Both forks pass through relatively narrow, steep-walled valleys (Williams et al. 1975). Timber harvest and other habitat alterations changed the hydrology of the greater Stillaguamish basin, such that floods that formerly occurred an average of once every 20 years now occur an average of every two years, degrading fish spawning and rearing habitat through scouring and silting (Eldridge and Killebrew 2008).

In response to habitat changes and harvest practices, Chinook salmon returns to the Stillaguamish River today are much reduced from the escapements documented earlier in the 19th century. Estimates for historic Chinook salmon returns ranged from 9,700 - 13,321 per year as compared to an average of 1080 in recent years (1996-2003) based on redd surveys. In the last few decades, the 12-year moving average for adult returns has been well below the 2,000 fish escapement goal agreed to by Washington Department of Fish and Wildlife, the Tulalip Tribes and the Stillaguamish Tribe (WDF 1977). Because of the depressed nature of the Chinook salmon populations in the Stillaguamish and other rivers in Puget Sound, these stocks were listed as threatened by the National Marine Fisheries Service (NMFS) in March 1999 under the Endangered Species Act (ESA).

There are two distinct native stocks of Chinook salmon recognized in the Stillaguamish basin (Figure 1). The more abundant is a summer-run stock spawning in the NF and its tributaries, which currently averages 1,048 fish a year based on redd surveys. The summer stock numbers dropped to historic lows (around 400 returning fish) in the mid-1980's and has been augmented annually by an integrated recovery hatchery program, implemented in 1987 (Eldridge and Killebrew 2008). A mixture of marked hatchery program fish and natural or wild-origin fish are spawned each year, the juveniles are reared and released, and returning hatchery-origin and wild-origin adults are allowed into natural spawning areas. Genetic testing has confirmed that program fish are indistinguishable from the wild-origin fish (Eldridge and Killebrew 2008). The Tribe has not had a directed Chinook salmon fishery in over 20 years.

The second Chinook salmon stock on the Stillaguamish is a fall-run stock which spawns in the SF and its tributaries, the mainstem of the Stillaguamish, and Pilchuck Creek, a tributary to the mainstem. The fall stock has declined precipitously in the last few years with the average run now barely over 100 fish based on redd surveys (WDFW, Peter Verhey, unpublished data). A

hatchery program was implemented in 2010 following the model of the summer-run program. The Tribe's smolt and spawner monitoring program provided data that formed the foundation for the tGMR project funded by the SSC.

Experimental design and field sampling

This project estimated the abundance of spawners in the Stillaguamish River (Figure 1) for brood year 2012 and compared estimates to brood years 2007 through 2011. The project used a genetic mark-recapture (GMR) protocol developed by Rawding et al. (2014) employing a pooled Peterson estimate (Seber 1982) to estimate spawner abundances. A standard mark-recapture estimates population abundance by marking and releasing individuals captured in a first sampling and in a subsequent sampling the proportion of marked (recaptures) to unmarked individuals provides the estimate for the population size. The estimate assumes marks are retained, marked and unmarked individuals have equal probability of capture, marked individuals are correctly identified and their behavior is unaltered, and the population is closed. In a genetic mark-recapture, individuals are "marked" by their genotype. In the parent-based tGMR described by Rawding et al. (2014), genotyped spawner carcasses are "marks", genotyped out-migrating juveniles are "captures" and juveniles that assign back to a spawner parent are "recaptures" of the parent's genotype.

Spawner tissues (fin clip or scale) were collected during scheduled weekly spawner surveys conducted in September and October. Surveys include the major spawning areas in the NF and SF. Most spawning takes place in the NF from the mouth upriver to rivermile (RM) 34.4, especially between RM 14.3 to 30.0. Spawning is also observed in the lower reaches of Boulder River, Squire Creek French Creek, Deer Creek, and Grant Creek. In the SF most spawning takes place in the mainstem and in Canyon, Jim and Pilchuck creeks. However, poor visibility and high flow make spawning surveys in the SF challenging.

Smolt samples are collected from February to July with an EG Solutions® screw trap on the mainstem at RM 6, downriver of the confluence of the NF and SF (Figure 1, see (Griffith 2011) for details of smolt trapping). In brief, smolt trap efficiency was calibrated using a standard mark-recapture technique: a known quantity of hatchery smolts were collected and marked with Bismark brown and released above the smolt trap. The capture of marked smolts per unit of effort provides the estimate for trap efficiency, roughly 1% in 2010 (Griffith 2011). The trap operates in 6 hour time windows stratified throughout each day of the week. Smolts are identified to species, enumerated, checked for tags and adipose fin-clips, biological measurements are made on a subset of hatchery and wild (natural-born) smolts, and fin clips are taken from wild (unclipped) smolts for genetic analysis. Roughly every other smolt fin clip was genotyped to match the number of juvenile samples outlined in the proposal (more juveniles were sampled than there were available funds).

Collections and genotyping

We genotyped Chinook salmon spawner carcass samples collected in 2012 (see Table 1 for all collections throughout project) and genotyped smolts sampled from a mainstem smolt trap in 2013. We also genotyped tissue samples collected from the 2012 hatchery broodstock to help identify unmarked hatchery juveniles (juveniles that left the hatchery without receiving an adipose fin clip). Smolts collected in the mainstem were assigned back to natural spawners and hatchery broodstock for the tGMR project described below.

Fish were genotyped at the 13 standardized GAPS microsatellite DNA loci. We added the locus Ssa-197 which has been useful for distinguishing Chinook salmon in the North and

South Fork Nooksack rivers, for 14 loci in a complete genotype (Table 2). Genomic DNA was extracted from tissue samples using silica membrane kits (Macherey-Nagel). Some smolt tissue samples were so tiny that a second elution was performed and DNA was concentrated by evaporating the sample. Microsatellite alleles were PCR-amplified using fluorescently labeled primers (see Table 2 for detailed PCR information). 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, and 1X Promega PCR buffer. The PCRs followed a "touch-down" protocol. After initial two minute denature at 94°, there were three cycles consisting of 94° for 30 seconds, annealing at 60° (temperature stepped down 1° each cycle) for 30 seconds, extension at 72° for 60 seconds. These were followed by 36 cycles consisting of 94° for 30 seconds, annealing at 50° for 30 seconds, extension at 72° for 60 seconds, then a final 10-minute extension at 72°. 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 from Applied Biosystems) and GeneMapper software (Applied Biosystems).

Genotyping was critical to the success of the project and genotyping errors could bias results. If a locus (or loci) amplifies poorly with degraded DNA, as is often the case for decayed spawner carcasses, there could be errors in spawner genotypes. Genotyping errors also arise from artifacts in the genotypic data or weak amplifications and such errors could prevent offspring from assigning to their true parent. To minimize scoring errors, we repeated the PCR for poorly amplifying DNA using lab conditions for difficult DNA. If warranted, we also repeated DNA extraction and PCR. For all data, two people scored genotypes independently and reconciled any scoring differences. We set a data threshold of 10 or more loci in a genotype to maximize assignment power and minimize spurious assignments.

We used the software programs FSTAT (Goudet 1995) and GENETIX (Belkhir et al. 2001) to calculate genetic statistics for collections. These statistics include conformation to Hardy-Weinberg equilibrium (HWE) expectations, heterozygosity, genetic diversity (using FSTAT) and linkage disequilibrium (using GENETIX). The HWE and genetic diversity measures (heterozygosity - does a locus have two different alleles), provide information on genotyping error (missed weak-amplifying alleles), sampling errors (sampled two populations or a family rather than a random sample from a single population), and population conditions (low population size and inbreeding). Allelic richness - average number of alleles per locus, corrected for different sample sizes – is also a genetic diversity measure that informs about population conditions. Linkage disequilibrium is another clue to non-random sampling; a sample with family members will have several representations of the parental allele combinations such that the loci appear to be linked or situated on the same chromosome.

Genetic Mark Recapture

The genetic mark-recapture (GMR) analysis was conducted in three stages: 1) genotype smolts and spawners, 2) assign smolts to spawner parents, and 3) use data in the mark-recapture equations to estimate abundance. We used two estimators for the tGMR, a binomial estimator that allowed all recapture data regardless of whether the spawner had been previously recaptured in a sibling juvenile, and a hypergeometric estimator that used only unique recaptures (both described in more detail below). Because each stage in the tGMR had complications that potentially biased results (e.g. assignment error, unmarked hatchery juveniles, yearling juveniles, juvenile sampling disproportional to out-migration), we developed methods to minimize complications and assess possible biases (described below).

After genotyping, we assigned juveniles to potential parents in pedigree analyses using the programs FRANz (Riester et al. 2009) and COLONY (Wang 2004, 2007, Wang and Santure 2009). Both programs use maximum likelihood to assign offspring to parents but differ in other respects. For our purposes, we began with FRANz because it assigns parents within minutes with an efficient algorithm, whereas the method employed in COLONY takes days to weeks (time depends on the size and relatedness of the data set and settings chosen for the analysis). FRANz considers the genotypes for a potential parent-offspring pair (or triad) and compares the likelihood that they are a one parent-offspring pair or two parent-offspring triad versus the likelihood that they are unrelated (calculates the logarithm of the odds or LOD score), allowing a user-specified number of mismatches among genotypes in the pair and triad. We used a simulation program in FRANz to guide the number of acceptable mismatches in pairs and triads. FRANz simulated 100,000 parent-offspring pairs, 100,000 parent-offspring triads, and 100,000 unrelated pairs and triads. FRANz calculated the number of mismatches per pair and per triad of related individuals, providing an estimate of expected mismatches among related individuals in our data set. We compared these to the mismatch distributions for 100,000 unrelated pairs and triads for estimates of mismatches among unrelated individuals. Based on these results, we accepted a parent-offspring pair with 0 or 1 mismatch. We accepted a triad with up to 2 mismatches if there was 1 mismatch with each parent and not 2 mismatches to one parent and 0 to the other. The FRANz results were used for an initial examination of the data and a preliminary estimate for the tGMR.

The program COLONY was our main assignment program. COLONY uses maximum likelihood to construct full- and half-sibling family groups among juveniles and assigns parents to the full-sibling families. If parents are unsampled, COLONY constructs the hypothetical parents for sibling families. As mentioned, COLONY runs can take weeks to complete but supply information, such as unsampled parents, that is unavailable from FRANz. COLONY has four options for run length (short, medium, long and very long), three options for analysis (full likelihood, pair likelihood and full-pair likelihood), and three options for precision (low, medium and high). In initial data exploration, we ran short runs with full-pair likelihood and high precision to strike a balance between obtaining results in a timely manner and consistency in the results. Initial runs included hatchery broodstock in the parent pool in order to identify unmarked hatchery juveniles. In subsequent runs, suspected hatchery juveniles were excluded and only spawners collected on spawning grounds were included for parents. We ran the COLONY analyses multiple times and compared results. The final run was medium length with full-pair likelihood and high precision.

We compared results from FRANz and COLONY and used the FRANz results for binomial Lincoln-Peterson calculations following Seber (1982):

$$N_{bin} = \frac{M(C+1)}{(R+1)} \qquad (1)$$

where N_{bin} = adult escapement based on the binomial model, M= marks - adult carcasses that were successfully genotyped, C= captures - natural origin smolts that were captured at the smolt trap and successfully genotyped, and R= recaptures – carcasses assigned to a juvenile through parentage analysis (1 if the recaptured juvenile is assigned to a single genotyped parent and 2 if the recaptured juvenile is assigned to two genotyped parents.

This has a binomial distribution that allows sampling with replacement - all juvenile data can be used regardless of whether juveniles share the same parent (resampling). We estimated variance using a Bailey's approximation (Bailey 1951, Seber 1982):

$$\text{var}(N_{bin}) = \frac{M^2(C+1)(C-R)}{(R+1)^2\,(R+2)} \quad (2)$$

We used COLONY results to estimate spawners based on Chapman's approximation to the hypergeometric estimator. The hypergeometric is based on sampling without replacement and thus uses only unique parent assignments (no juveniles with shared parent, just the first sampling of the parent). For the hypergeometric estimation, the input values to the calculation were the number of unique parents and number of unique assignments to sampled parents (*M* = genotyped carcasses, *C* = unique number of parents, and *R* = unique assignments):

$$N_{hyp} = \frac{(M+1)*(C+1)}{(R+1)} - 1 \quad (3)$$

The variance for the hypergeometric estimator was estimated using the following equation:

$$\text{var}(N_{hyp}) = \frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2(R+2)} \quad (4)$$

For a third method to estimate spawners, we used rarefaction to infer the number of parents that produced the juveniles sampled (effective number of breeders, N_b) based on the estimated families encountered. We used COLONY sibship estimates for rarefaction curves that described the unique number of breeders giving rise to the juvenile sample, following Petit and Valiere (2006). This approach uses information from all juveniles sampled to make inferences about the population of spawners. While the binomial and hypergeometric provide total spawner abundance estimates, the rarefaction curve estimates the number of successful breeders. We used an R script (T. Seamons, WDFW) that randomly resampled increased-sized subsets of the juvenile data set 10,000 times for each sized subset and calculated the unique number of spawners for each resampling. The R script employed the Beverton-Holt (BH) spawner-recruit model (Beverton and Holt 1956) and Continuous Smooth Hockey Stick (CSHS) model (Froese 2008) to generate rarefaction curves that reached asymptotes at the maximum estimates of unique breeders and generated confidence intervals for these estimates.

Potential sources of bias

Unmarked hatchery juveniles were one potential source of bias. In 2013, less than 1% of juveniles left the hatchery with their adipose fin intact (un-clipped) (Kip Killebrew, Stillaguamish Tribe Fisheries Biologist, unpublished data). Unidentified yearling out-migrants are another potential source of bias, because in the Stillaguamish River a few juveniles outmigrate as yearlings. Both un-clipped hatchery juveniles and undetected yearlings would inflate "capture" number and thus inflate abundance estimates because their parents were absent from the "marked" group and these juveniles have no chance of assigning to their parents. To

correct for this, we genotyped hatchery broodstocks and identified hatchery-origin juveniles by assigning them to hatchery parents using FRANz and COLONY. Juveniles assigning to hatchery parents were removed from the analysis. To identify potential yearling juveniles, we plotted juvenile length versus out-migration week and identified yearlings as juveniles that were over 25% larger than other juveniles caught in the same week and removed suspected yearlings from analyses. We estimated spawner abundance in several ways and compared how different assumptions and corrections affected calculated abundance values and compared these values to expanded redd-based estimates.

Estimate spawner abundance by hatchery or natural origin

Tissue samples were obtained from both hatchery- and wild (natural)-origin spawners (Table 1). Because all hatchery fish are mass marked, we estimated the proportion of hatchery spawners (pHOS) using the following equation:

$$pHOS = H_{carc}/T_{carc} \quad (5)$$

where H_{carc} is the total number of hatchery carcasses samples and T_{carc} is the total number of carcasses sampled. The variance for this proportion was estimated as:

$$var(pHOS) = (pHOS)(1-pHOS)/(T_{carc} -1) \quad (6)$$

The proportion of natural orgin spawners (pNOS) = 1 – pHOS and the Var(pNOS) = Var(pHOS). The number of Hatchery Origin Spawners (HOS) is estimated by:

$$HOS = pHOS * N \quad (7)$$

where N is the escapement estimate.

The number of natural origin spawner (NOS) is estimated by:

$$NOS = pNOS * N \quad (8)$$

The variance for HOS was estimated by:

$$var(HOS) = N^2var(pHOS) + pHOS^2var(N) - var(pHOS)var(N) \quad (9)$$

and the variance for NOS was estimated by:

$$var(NOS) = N^2var(pNOS) + pNOS^2var(N) - var(pNOS)var(N). \quad (9)$$

We also examined the influence of spawner status (hatchery or wild) on the number of offspring produced in a generalized linear model (GLM). We included fork length, sex, hatchery or wild status, and recovery date as factors in the GLM and used the glm.nb package in R to run the analysis.

Results

Collections and genotyping

Genotype abundance for juveniles and carcasses varied among brood years (Table 3). We had mixed success in genotyping spawner scale samples; success per collection ranged from <1% to 40%, averaging around 14% (Table 1). Scales from decayed carcasses often produced low quantities of degraded DNA which usually failed to amplify. Samples with less than 10 loci were eliminated from the study to maintain assignment power for the tGMR. Genotyping success was higher for spawner tissue samples (rather than scales), and averaged around 73%. Success was higher because spawner survey crews targeted spawners that were in the best condition (freshest carcasses) for tissue sampling. Genotyping success for hatchery broodstock tissue samples was 100% for 2012 broodstock (following the same protocol of reruns and re-extractions when samples failed). Success was highest for broodstock samples because they were collected from living fish rather than decayed carcasses. Genotyping success was also high for juvenile samples, but there were still some juveniles each year without genotypic data. Less than 10% of juvenile samples (eg. 11/1142 in 2012) collected each year in the mainstem trap were likely to be another species because they either failed to amplify at the Chinook salmon locus suite or their alleles were outside the size range for Chinook salmon (juvenile salmonids of different species can look similar). Genetic statistics indicated that most spawner collections were random samples (Table 4), with the exception of the wild-origin spawners collected in 2012 that deviated from Hardy-Weinberg equilibrium (HWE) expectations overall, due to disequilibrium at one locus. Genetic statistics also indicated that juvenile collections included families: linkage disequilibrium was high after correcting for multiple tests.

Identifying unmarked hatchery juveniles and yearlings

We identified unmarked hatchery juveniles in each collection year (Table 1, two among 2013 juveniles) and confirmed these putative hatchery juveniles had no parents among the natural spawners by conducting assignment tests without hatchery broodstocks in the parent pools. Because in some years some of the hatchery broodstock were below our 10 locus data threshold, our method potentially missed some unmarked hatchery juveniles. However, we had genotypes from 100% of the 2012 broodstock. Further, tGMR results suggested that unmarked hatchery juveniles had minimal impact on *N* calculations (presented below).

The regression plot suggested that there were 10 putative yearlings mixed in with sub-yearlings (Figure 4) and these fish were excluded from the analysis.

Genetic Mark Recapture

Similar to Small et al. 2012, the number of assignments using FRANz and COLONY were congruent. Because the programs used different methods to assign parents (matching parent-offspring genotypes versus constructing families from pair-wise sib-ships, respectively) there were minor differences between number of juveniles assigned to a parent as well as minor differences among which juveniles were assigned to a parent. FRANz assigned more parents than COLONY, likely because COLONY restricted some assignments with genotypes created for unsampled parents.

Spawner estimates

Similar to previous brood year estimates, (Figure 2) the 2012 brood year estimated N value was slightly higher when unmarked hatchery juveniles and yearlings were included

(hatchery juveniles and yearlings increased capture numbers). But there were few hatchery juveniles and yearlings so the difference was minimal and confidence intervals encompassed N calculated without hatchery juveniles and yearlings. Unmarked hatchery juveniles and yearlings appeared to impart a minimal bias to abundance calculations – N calculated without removing suspected unmarked hatchery juveniles and yearlings was <1% higher than N calculated after their removal.

The hypergeometric estimate differed from the binomial estimate in that it was based on sampling without replacement. For the hypergeometric, we ran COLONY to estimate total number of unique parents (sampled and unsampled) that gave rise to the juvenile data set. The unique number of parents was our capture value and the total number of unique assignments to sampled parents was our recapture value. Unique among all brood years, the hypergeometric estimate for 2012 brood year was virtually the same as the binomial estimate (Table 5, Figure 2).

Rarefaction curve

Rarefaction curves estimated the successful number of breeders (fish producing returning offspring), rather than the total escapement (fish in spawning areas). If reproductive success is unequal, which is usually the case for naturally-spawning salmonids, there will be fewer successful breeders than actual spawners. We thus expected the rarefaction curve estimate to be less than tGMR estimate. In estimates from 2007, 2008, 2010, and 2012 (Figure 3), the point estimates from the rarefaction curves were far below the binomial estimates (Table 5). For the 2009 and 2011 brood years, the bounds for the Beverton-Holt (BH) rarefaction curve estimates encompassed the estimates from the binomial (Table 5). In all years the BH estimates were higher than the Continuous Smooth Hockey Stick (CSHS) estimates (Figure 3).

Spawner origin, abundance and reproductive success

Assuming carcass recoveries were unbiased by origin, the pHOS estimate was 41.5% (95%CI = 33.9% - 49.0%) and pNOS estimate was 58.5% (95%CI = 50.1% - 66.1%). Using the binomial distribution, the estimate for HOS was 725 fish (95%CI = 576 - 875) and the estimate for NOS was 1024 fish (95%CI = 869 - 1180).

Parent recaptures indicated that the hatchery-origin spawners produced fewer offspring than wild-origin spawners (Table 6). However, when controlling for factors including fork length, sex and collection date, the generalized linear model showed that the difference in reproductive success between hatchery and wild spawners was not significant ($p = 0.9$). As expected for salmonids, reproductive success was positively correlated with size. Reproductive success was also negatively correlated with sex: on average, males had fewer offspring than females, but the difference was not significant.

Estimate redd count expansion factor

We had a total of six years of tGMR and redd-based estimates. Graphical analysis of the data showed that the redd-based estimates were consistently lower than the binomial and hypergeometric estimates (Figure 5 and 6). There was more variability in the comparison of the binomial estimates with the redd-based estimates than in the comparison of the hypergeometric estimates with the redd-based estimates. Brood year 2007 had a CV greater than 28% and redds were estimated using Area Under the Curve, rather than full basin survey as in subsequent years, so this data point should be interpreted cautiously. If brood year 2007 is excluded, the redd-based estimates explained 80% of the variance in the binomial estimates and 67% of the variance in the hypergeometric estimates. If brood year 2007 is included, the redd-based estimates

explained 10% and 58% of the variance in the tGMR estimates, respectively. We conducted an exploratory analysis of correlations between redd-based escapement estimates and survey conditions (survey frequency, percentage of basin surveyed, environmental conditions), and mark-recapture estimates. The exploratory analysis suggested that survey covariates were uninfluential in the relationship between redd- and genetic-based estimates. A calibration factor analysis will be completed for the final report in 2015.

Discussion

Chinook salmon spawner abundance is a key parameter the Stillaguamish River that impacts management decisions and fisheries actions in North Puget Sound. However, estimating abundance for Chinook salmon is challenging because the Stillaguamish River is large and often turbid and some spawning areas are inaccessible to humans. Spawners enter the river in fall when storms often create conditions that further obscure visibility. Enumerating spawners directly can be difficult and abundance estimates have been based historically on expanded redd counts. Yet, the same factors that make enumeration challenging also make redd counts challenging. Here we present alternative methods for estimating spawner abundances based on mark-recapture theory.

A mark-recapture abundance estimate is based on five key assumptions (Seber 1982): marks are permanent, marks are correctly identified and reported, the system is closed (N is fixed), marking does not affect catchability, and all animals have the same probability of being tagged in the first sample, or caught in the second sampling, or marked fish mix uniformly with umarked fish (Schwarz and Taylor 1998). For tGMR, Rawding et al. (2014) noted that the equal catchability assumption must be modified to the assumption that all animals have an equal probability of being marked in the first sample or that the probability of capture in the second sample is independent of the first sample. Violating these assumptions bias the estimate of N and the direction and magnitude of the bias was determined by the violation. In this study we use individual spawner genotypes as the "mark" and use juveniles assigning to spawner parents as "recaptures" of the parent genotype in the second sampling. But for our estimates of N to be valid, we must meet the basic assumptions. Because conditions and sampling varied, we assessed how our methods may have violated the assumptions and may have biased N.

Our data meet some assumptions completely (system is closed if hatchery fish are detected and we use only offspring from the appropriate brood year) and violate other assumptions, with the violations varying by year. Because we use genotypes for the marks, these are permanent and do not affect catchability. However, Chinook salmon die after spawning and spawner carcasses often decay before sampling. Thus, some marks may have been incorrectly identified or unreported if the DNA from the tissue was of poor quality and yielded spurious genotypic data (Copeland et al 2009). An incorrect genotype for a spawner would preclude its recapture in its offspring because the offspring's genotype would not match its true parent and the juvenile would be incorrectly identified as offspring of unsampled parents. This would bias the N estimate upward. We corrected for this problem by limiting our data to samples with at least 10 loci scored in their genotype. In our experience, better quality DNA amplifies more consistently in the PCR and yields reproducible genetic data. A sample with at least 10 loci in its genotype indicates better quality DNA. However, this 10 locus threshold may have introduced an upward bias or increased uncertainty by increasing the number of unsampled parents.

Another way a mark may be incorrectly identified is by error in scoring genetic data. We corrected for this problem by having two independent data scores, resolving differences, and rerunning ambiguous or missing data. However, some alleles at some loci may amplify weakly such that a second allele is undetected even with careful scoring (allelic dropout) and the individual is scored erroneously as a homozygote rather than as a true heterozygote. Allelic dropout may be more pronounced with lower quantity DNA. To correct for this problem, we allowed up to one mismatch between a single parent and its offspring in genetic assignments, per Lukacs and Burnham (Lukacs and Burnham 2005). If our mismatch criterion is too stringent it would bias estimates higher than the true value because we would not assign juveniles to their sampled parent. If the mismatch criterion is too relaxed, it would bias estimates lower than the true value because we would accept an assignment of a juvenile to a parent that was not its true parent.

The equal catchability assumption is often difficult to meet in mark-recapture studies. As described in the methods section we scheduled equal sampling effort over the spawning period and in all major spawning areas to collect carcasses with the goal of having equal capture probability for all carcasses. However, in some years the offspring per spawner and carcass recoveries favored males and in other years favored females, although the difference in offspring per spawner was not significant for BY 2012. Rawding et al. (2014) proposed using the binomial model to stratify the estimates by sex if carcass recoveries or offspring per spawner are biased by sex. Further, if weather or other factors prevented regular spawner surveys, the probability of sampling some spawners would not be equal. In addition, differences in family size could also violate the assumption of equal catchability if spawners with more offspring were more likely to be recaptured in a random sample of juveniles. Our GLM analysis suggests that of the factors considered only spawner length may influence offspring per spawner or recapture probabilities. In this case, the hypergeometric tGMR estimator is likely to be less sensitive to this violation of this assumption, because the heterogeneity in individual capture probabilities is reduced by restricting the offspring to one per spawner. However, our abundance estimates based on the binomial and hypergeometric models were not significantly different suggesting that factors other than length may account for differences in offspring per spawner, such as number of mates (Richard et al. 2013).

Another possible source of bias could arise from sampling only unmarked, presumably natural-origin spawners for brood years 2007 to 2010 because natural origin spawners may have higher relative reproductive success than hatchery fish. However, Rawding et al. (2014) and Seamons et al. (2012) found no difference in relative reproductive success to the outmigrant stage between naturally spawning hatchery-origin and natural-origin Chinook salmon. Based on the GLM, we also found no difference in reproductive success between hatchery- and natural-origin spawners for brood years 2011 and 2012.

We addressed objective 1, estimate spawner abundance, the main focus of the original proposal. We also addressed objective 2, estimate natural spawner abundance by origin, natural or hatchery. Hatchery-origin spawners were more abundant and although their average reproductive output was lower than natural-origin spawners, the difference was not significant: hatchery- and wild-origin spawners contributed roughly equally to offspring production in the Stillaguamish River. We have described differences between mark-recapture and redd-based abundance estimates; some of the difference among estimates arose from poor environmental conditions when redd observations were less than 100% and redd-based estimates may be a minimum estimate in those cases.

Comparison among techniques

For each brood year, the binomial and hypergeometric estimates were similar (Figure 2) and differences were greatest when there were few recaptures (Table 3). An advantage of using the binomial estimator is that it uses all the data and may be preferred when carcass recoveries are biased by sex because the genetic abundance estimates could be stratified by sex (Rawding et al. 2014). However, if carcass samples were biased toward larger individuals that also produced more offspring, then mark recoveries would be inflated in the second sample (carcasses assigning to smolts), which would underestimate abundance (Arnason et al. 1996). Under these circumstances, the hypergeometric tGMR estimator is likely to be less sensitive to violations of the equal catchability assumption, because restricting offspring to one per spawner reduces the heterogeneity in individual capture probabilities. However, the hypergeometric estimator may be more susceptible to bias because unique recaptures are always less than total recaptures and mark-recapture estimates are biased when recoveries are very low (Seber 1982).

We compared results from binomial and hypergeometric estimates with estimates from expanded redd counts and rarefaction curves (Figure 2, Table 5). The binomial, hypergeometric, and redd count expansion estimated census size (successful breeders plus spawners that produced no offspring) and the rarefaction curve estimated successful breeders. In all years the CI's overlapped between the binomial and hypergeometric estimates and the redd-based estimate mostly aligned with either or both estimates. Discrepancies could arise from sampling biases impacting binomial and hypergeometric estimates and from bias introduced in redd counts (discussed below). For the rarefaction curves the BH estimates were always higher than the CSHS estimates because the BH estimate is based on the assumption of unlimited juveniles. In reality, carrying capacity of the Stillaguamish system limits the number of juveniles and the BH model likely overestimates the number of successful breeders (Petit and Valiere 2006). We thus recommend the CSHS model unless very few juveniles are collected. The rarefaction curve (CSHS) breeder estimate was lower than the census size estimates in all years. This lack of concordance between breeder and census estimates is expected in salmonids because reproductive success is unequal and there are fewer successful breeders than total breeders.

Discrepancies among estimates could also be related to turbidity and discharge. For example, in 2007 high water challenged redd counting during all of October, which likely led to an underestimate in the redd count. Further, sustained high flows flushed carcasses from the system making carcass recovery difficult, resulting in few genetic "marks" for that year (Table 3, Figure 5 and Figure 6). In years with better weather during spawning season, more carcasses were captured and discrepancies among estimates were smaller. In the 2008 brood year the point estimates from the tGMR methods were similar to each other and the smaller confidence intervals encompassed the redd-based estimate. In the 2009 brood year there was concordance between tGMR estimates and redd-based estimates, confidence intervals were small, and the CV was 5%. In the 2010 brood year, lower proportions of spawners and juveniles were sampled, tGMR estimates varied, confidence intervals were larger, and the CV was 15%, which was high but within the SSP goals. In the 2011 and 2012 brood years, high proportions of spawners and juveniles were sampled, but tGMR estimates were larger than the redd-based estimate, although estimates were closer in 2012. Among all years, sampling was best for the 2009 brood year with a high number of marks, captures and recaptures. The tGMR estimate for 2009 (~1100 spawners) suggested that at least 10% of the estimated number of spawners had been sampled (147 marks). The second best sampling was for the 2012 brood year and the tGMR estimate

(~1750 spawners) suggested that at least 9% of the estimated number of spawners had been sampled (164 marks).

Impact of hatchery and yearling juveniles

The hatchery program releases juveniles near middle April, roughly half-way through the wild juvenile out-migration period (see Appendix 1). Few (< 2%) are unmarked and less than 1% of the total outmigrating smolts (hatchery plus wild) are captured in the smolt trap. Because of abundant hatchery food and warmer water temperatures, hatchery juveniles tend to be larger at release than natural-origin juveniles of the same age. But hatchery and wild juvenile size distributions overlapped each other such that we were unable to identify hatchery-origin juveniles just by size and instead relied on assignments to hatchery broodstocks to identify unmarked hatchery juveniles. However, results suggested that unmarked hatchery juveniles had little impact on tGMR abundance estimates. We also considered how yearlings might affect results. Our efforts may have overlooked some yearlings because it may be impossible to distinguish them from sub-yearlings based solely on size (Mara Zimmerman, WDFW, pers. comm.) and require scale data for definitive identification. Yet, because yearlings are rare and the analyses are fairly robust to small variations in capture numbers (point estimates would vary slightly within nearly the same confidence interval), undetected yearlings are likely to minimally impact estimated *N*.

Conclusions

Our data suggests that tGMR is a useful tool for estimating Chinook salmon escapement in the Stillaguamish River. Based on binomial sampling, the tGMR estimates met CTC standards for precision in five of six years and four of six years for the hypergeometric model. Given that juvenile sampling efforts are standardized, increasing adult tissue sample collections is likely to meet precision standards. The CTC standards for unbiased estimates were largely achieved although one concern remains regarding the assumptions of equal capture probability and that marks were correctly identified and reported. WDFW is pursuing simulations and double sampling to address these concerns. In addition, increases in adult sample sizes and improvements in sample freshness have increased the number of genetic assignments.

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Table 1. List of samples in Genetic Mark Recapture analyses with number of samples analyzed (N) and the number of samples with at least 10 loci in their genotype (N>9 loci). Samples analyzed for the 2012 brood year study are in bold type.

WDFW code	Collection Location and run type (if known)	Life stage	N	N >9 loci	hatchery	yearling
07NI	North Fork Stillaguamish summer (spawning ground)	adult	74#	30		
08LC	North Fork Stillaguamish summer (spawning grounds)	adult	11	7		
08HX	North Fork Stillaguamish summer (spawning grounds)	adult	149#	57		
09CS	North Fork Stillaguamish summer (spawning grounds)	adult	152	114		
09NB	North Fork Stillaguamish summer (spawning grounds)	adult	139#	11		
10DD	North Fork Stillaguamish summer (spawning grounds)	adult	70	52		
10NW	North Fork Stillaguamish summer (spawning grounds)	adult	12#	0		
11KJ	North Fork Stillaguamish summer (spawning grounds, wild and hatchery)	adult	141	109		
11KJ	North Fork Stillaguamish summer (spawning grounds, wild origin)	adult	56#	2		
11NP	North Fork Stillaguamish summer (spawning grounds, hatchery origin)	adult	11#	1		
12CP	**North Fork Stillaguamish summer (spawning grounds, wild and hatchery)**	**adult**	**217**	**163**		
12CP	**North Fork Stillaguamish summer (spawning grounds, wild and hatchery)**	**adult**	**111#**	**1**		
07NK	North Fork Stillaguamish summer (hatchery broodstock)	adult	155	94		
08NA	North Fork Stillaguamish summer (hatchery broodstock)	adult	129	83		
09CS	North Fork Stillaguamish summer (hatchery broodstock)	adult	143	107		
10DC	North Fork Stillaguamish summer (hatchery broodstock)	adult	151	135		
11BO	North Fork Stillaguamish summer (in-season, hatchery broodstock)	adult	169	167		
12CL	**North Fork Stillaguamish summer (hatchery broodstock)**	**adult**	**155**	**155**		
08LE	Mainstem Stillaguamish	smolt	567	508	14	6
09CQ	Mainstem Stillaguamish	smolt	799	751	8	5
10DA	Mainstem Stillaguamish	smolt	1315	1232	33	5
11BU	Mainstem Stillaguamish	smolt	597	544	29	0
12CO	Mainstem Stillaguamish	smolt	1520	1461	25	1
13BE	**Mainstem Stillaguamish (subsample from 1407 juveniles)**	**smolt**	**1142**	**1109**	**2**	**10**

[#] scale samples

Table 2. Information for multiplexes and loci including annealing temperature (°C) primer concentration, and size range of GAPS alleles (in basepairs). References for primer sequences are under Citation.

				GAPS standardized loci		
Multiplex	Locus	Anneal temp	conc [uM]	Alleles	Size Range	Citation
Ots-M	Ots201b		0.35	37	133-342	Banks, Oregon State University, unpublished
	Ots208b		0.2	30	142-378	Grieg et al. 2003
	Ssa408		0.2	20	180-320	Cairney et al. 2000
Ots-N	Ogo2	60	0.15	15	200-258	Olsen et al. 1998
	Ssa197[a]		0.25	39	171-318	O'Reilly et al. 1996
Ots-O	Ogo4	56	0.18	14	132-170	Olsen et al. 1998
	Ots213		0.18	37	178-378	Grieg et al. 2003
	OtsG474		0.16	11	144-220	Williamson et al. 2002
Ots-R	Omm1080	53	0.26	41	162-458	Rexroad et al. 2001
	Ots3M		0.12	12	122-170	Banks et al. 1999
Ots-S	Ots212		0.3	27	123-263	Grieg et al. 2003
	Ots9		0.1	6	99-115	Banks et al. 1999
Ots-T	Oki100	50	0.37	32	164-353	Miller, Department of Fish and Oceans, unpublished
	Ots211	60	0.2	27	196-337	Grieg et al. 2003

[a] We collect data for this locus in multiplex Ots-N, but Ssa197 is not a GAPS locus.

Table 3. Escapement estimates for Stillaguamish River Chinook salmon for brood years 2007-2012 using transgenerational genetic mark-recapture (tGMR) based on the binomial model. Genotyped carcasses are under "Marks", genotyped outmigrating juveniles are under "Captures", juveniles assigned to a spawner are under "Recaptures", and the coefficient of variation for the tGMR estimate is under "CV". Redd-based abundance estimates were from expanded redd counts. tGMR estimate is from pooled binomial estimate and excluded unmarked hatchery juveniles and yearlings.

Brood Year	Genotyped carcasses (Marks)	Genotyped juveniles (Captures)	Juveniles assigned (Recaptures)	GMR Estimate	GMR CV	Redd-based estimate
2007	34	488	16	1954	23%	616
2008	72	744	55	1914	13%	1671
2009	147	1194	330	1061	5%	1001
2010	54	515	40	1358	15%	783
2011	112	1435	238	1345	6%	1017
2012	164	1109	207	1750	7%	1534

Table 4. Genetic statistics include gene diversity (Gene Div, expected heterozygosity corrected for collection size), allelic richness (Rich, average number of alleles per locus corrected for collection size of 22), and the departure from Hardy-Weinberg equilibrium expressed by F_{IS}, and associated *p* value for heterozygote deficit or excess. Underlined *p* value indicated significant departure from equilibrium. The number of linked pairs of loci were the number of locus pairs where 5% (or 1%) of 500 permuted values were larger than the actual value. Both hatchery- and wild-origin spawners were collected in the North Fork Stillaguamish River spawners in 2011 and 2012, and statistics were calculated for the spawner groups as a whole and for spawners grouped by hatchery and wild origin. South Fork Stillaguamish River spawner collections were too small for meaningful calculation of genetic statistics.

					heterozygosity			
					deficit	excess	linkage (91 pairs)	
	N	Gene Div	Rich	F_{IS}	*p* value	*p* value	5%	1%
07NF spawners	30	0.8741	14.79	-0.005	0.597	0.404	5	0
08NF spawners	64	0.8672	14.32	0.002	0.440	0.561	3	1
09NF spawners	125	0.8731	13.99	-0.009	0.844	0.156	24	8
10NF spawners	52	0.8654	14.29	0.002	0.443	0.557	3	1
11NF spawners	112	0.8696	14.15	-0.007	0.800	0.201	13	2
11NF_h_spawners	65	0.8721	14.21	-0.022	0.973	<u>0.028</u>	15	2
11NF_w_spawners	47	0.8669	14.02	0.013	0.168	0.833	6	0
12NF spawners	164	0.8766	14.39	0.015	0.019	0.981	17	5
12NF_h_spawners	68	0.8666	13.83	-0.011	0.839	0.161	12	3
12NF_w_spawners	96	0.8811	14.62	0.031	<u>0.001</u>	0.999	8	3
07NF broodstock	94	0.8727	14.11	-0.007	0.737	0.264	7	1
08NF broodstock	83	0.8661	14.18	0.005	0.322	0.679	10	3
09NF broodstock	107	0.8696	13.98	-0.012	0.909	0.091	15	2
10NF broodstock	135	0.8703	13.88	0.005	0.258	0.743	12	4
11NF broodstock	167	0.8746	14.13	0.001	0.435	0.566	34	17
12NF broodstock	155	0.8771	14.09	-0.009	0.887	0.114	15	3
08 mainstem smolts	488	0.8736	14.51	-0.002	0.652	0.351	85	70
09 mainstem smolts	738	0.8764	14.44	0.002	0.303	0.699	89	77
10 mainstem smolts	1194	0.8717	14.02	-0.001	0.565	0.437	91	83
11 mainstem smolts	515	0.8764	14.41	-0.008	0.982	<u>0.018</u>	88	69
12 mainstem smolts	1437	0.8735	14.27	0.004	0.071	0.930	87	70
13 mainstem smolts	1109	0.8719	14.49	0.008	<u>0.003</u>	0.997	84	62

Table 5. Summary of abundance estimates for all brood years using different methods (see Figure 3 for CSHS rarefaction curve values).

		all juv in	no H juv	hypergeometric	rarefaction curve	redd
2007	*N*	2157	1954	1291	327	616
	Var	269340	208427	137994		
	CV	24.06%	23.36%	28.77%		
2008	*N*	1935	1914	1711	496	1671
	Var	63244	61881	96936		
	CV	12.99%	12.99%	18.20%		
2009	*N*	1100	1061	1239	726	1001
	Var	3158	2921	6888		
	CV	5.11%	5.09%	6.70%		
2010	*N*	1508	1358	837	359	783
	Var	54804	42156	17076		
	CV	15.53%	15.12%	15.62%		
2011	*N*	1369	1345	1637	899	1017
	Var	7168	6914	10465		
	CV	6.19%	6.18%	6.25%		
2012	*N*	1769	1750	1787	947	1534
	Var	13577	13273	18903		
	CV	6.59%	6.58%	7.69%		

Table 6. Reproductive success by sex and by spawner group (by2012) in upper portion of table and generalized linear model results in lower portion of table. For reproductive success, the numbers of carcasses for the different status groups (H = hatchery-origin and W = wild-origin) are indicated by "N_{adult}" and the numbers of offspring are indicated by "N_{off}". The generalized linear model for reproductive success was based on fork length, sex, hatchery or wild origin and collection date. The "z value" is a test statistic that the estimated parameter is not equal to zero and is followed by the two-tailed probability (Pr(|>z|) for the z value. Bold values were significant, df = 163, residual df = 159.

		sex	
status		M	F
H	N_{adult}	28	40
	tot N_{off}	20	63
	avg N_{off}	0.71	1.58
W	N_{adult}	39	57
	tot N_{off}	42	82
	avg N_{off}	1.08	1.44

GLM

	Estimate	Std. Error	z value	Pr(>|z|)
fork length	**0.00358**	0.00116	3.086	**0.00203**
sex	-0.18786	0.27199	-0.691	0.48975
Origin (H v W)	-0.02247	0.24586	-0.091	0.92717
collection date	-0.00216	0.01138	-0.190	0.84919

Figure 1. Map of the Stillaguamish basin in relation to the greater Puget Sound (from Dale Gombert, WDFW).

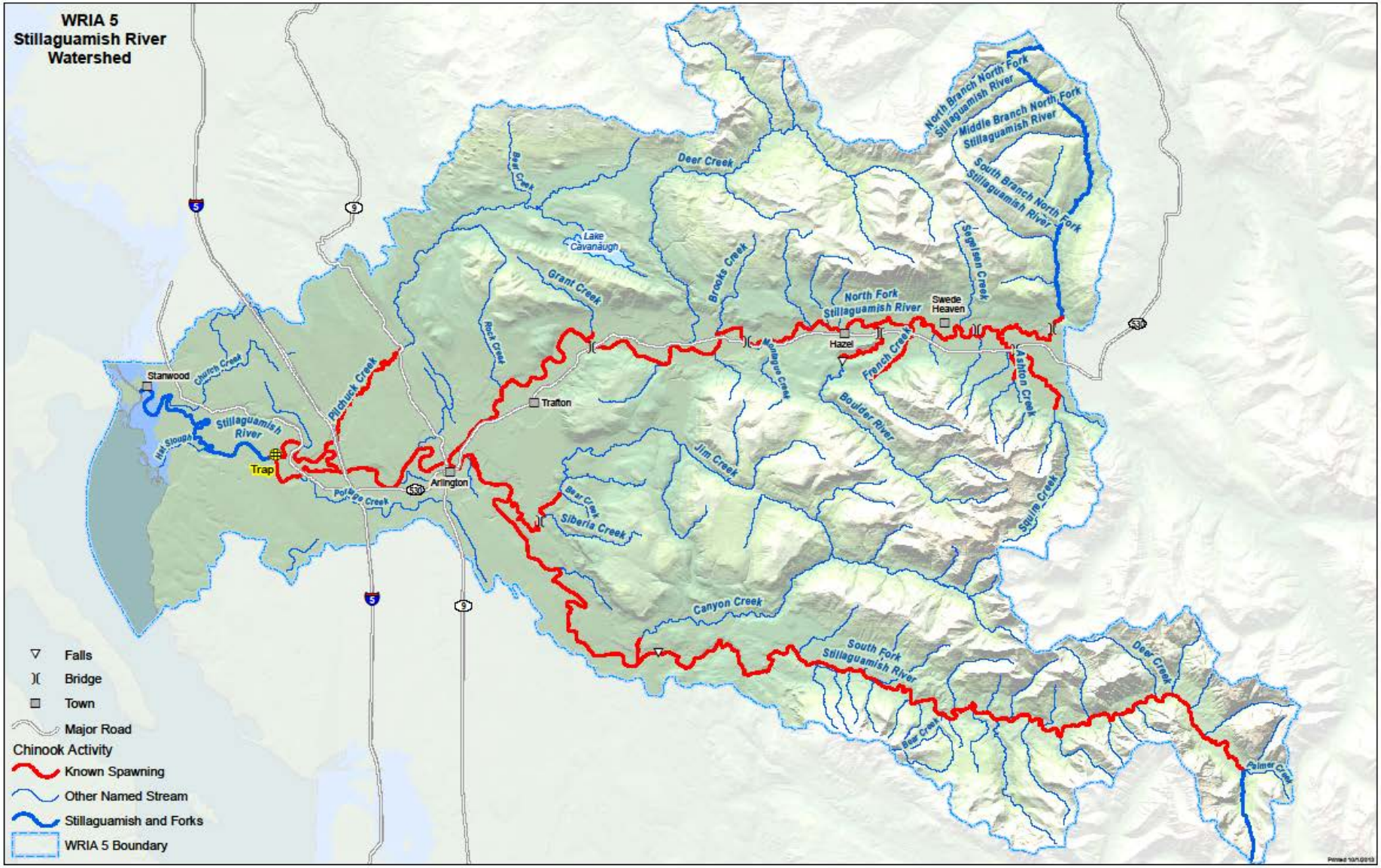
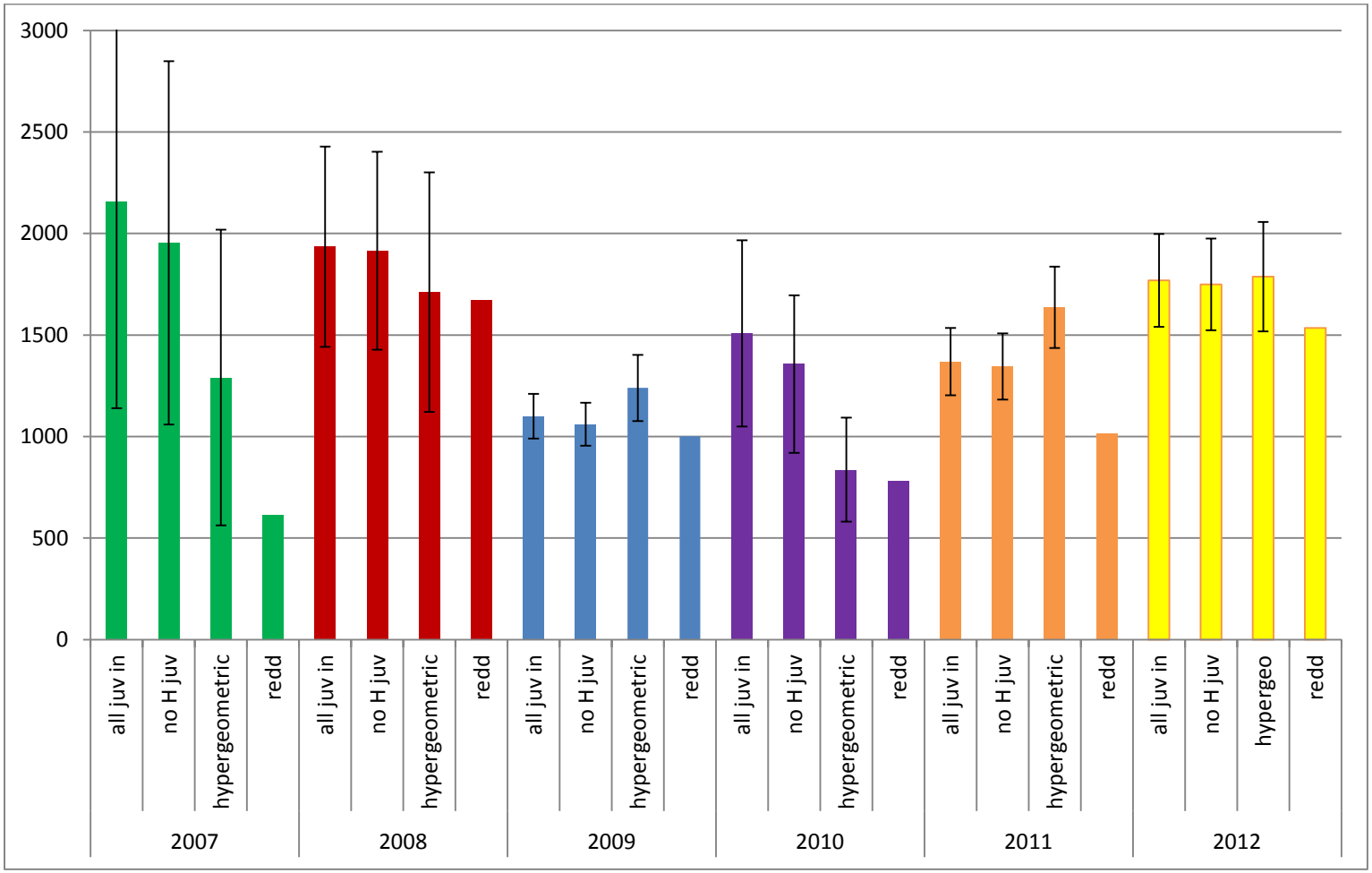


Figure 2. Comparison of the abundance estimates with different methods: all juveniles without removing hatchery and yearling juveniles (all juv in), removing hatchery and yearling juveniles (no H juv), hypergeometric with hatchery and yearling juveniles removed (hypergeometric), and redd-based estimate (redd). Actual confidence interval for the 2007 "all juv in" extended to 3174.



* binomial estimates (no H juv) presented in **Table 3.**

Figure 3. Rarefaction curves estimating spawners in brood years 2007 through 2012. The large dots are the averages over 10,000 re-samples.

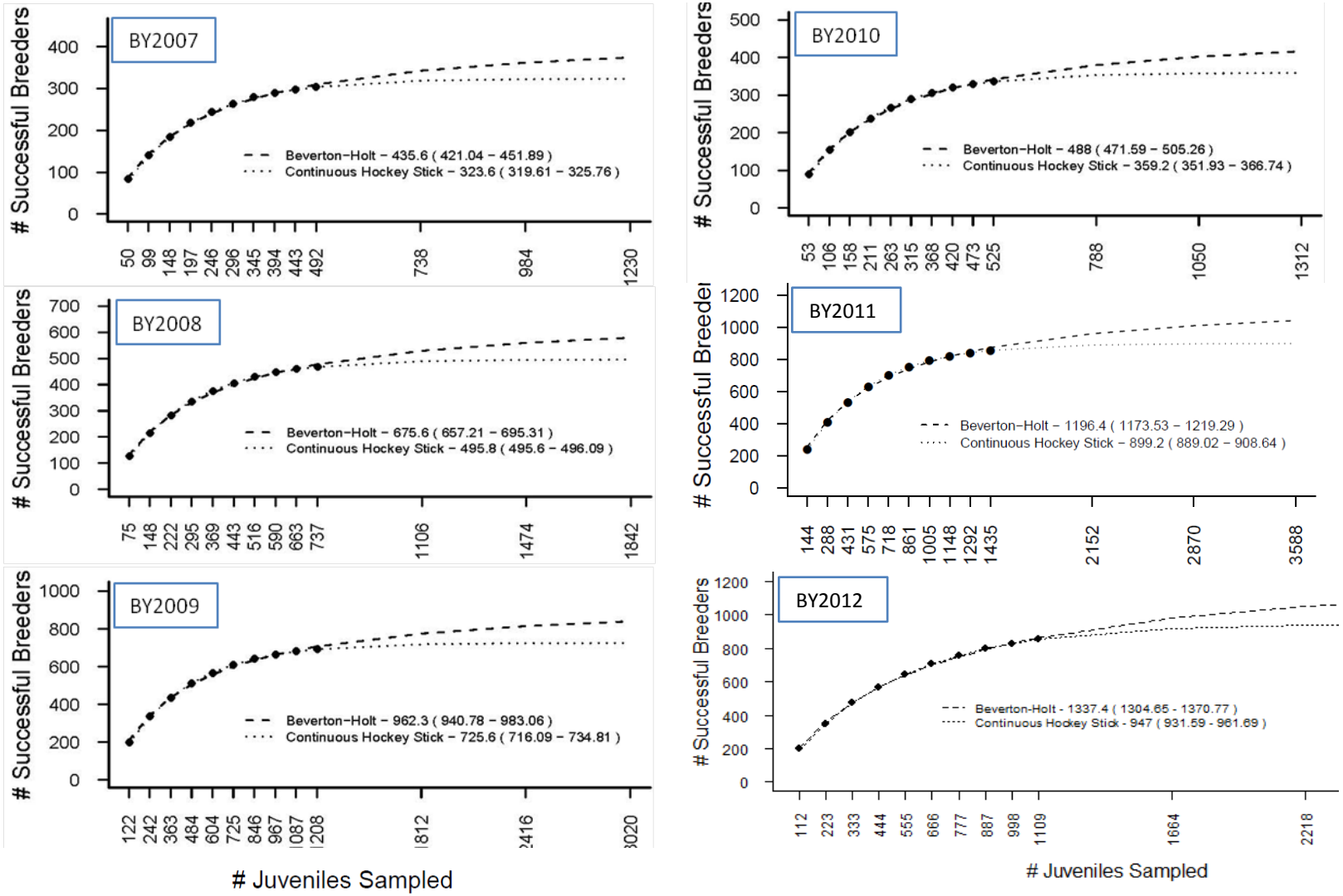


Figure 4. Plot of unclipped smolt lengths versus out-migration week. Yearlings are in red and were identified based on graphical analysis.

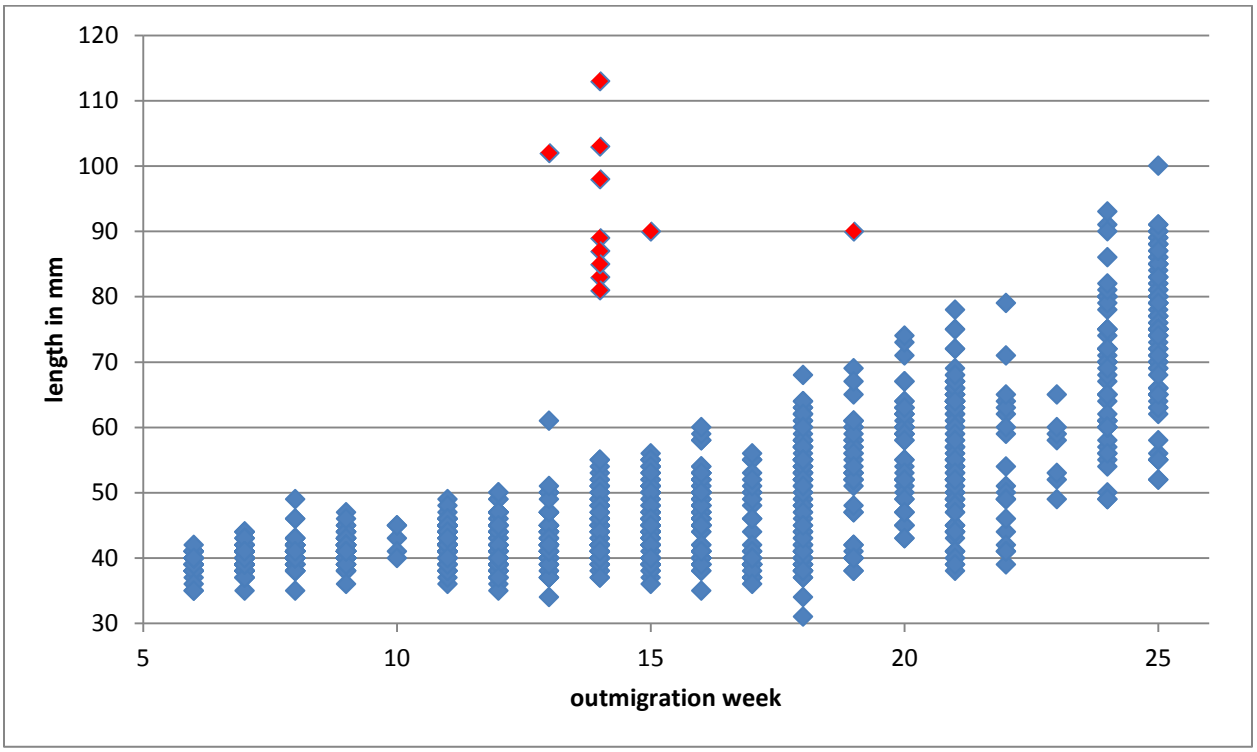


Figure 5. Comparison of binomial tGMR estimates with redd-based estimates. Inset to right includes data from 2007 brood year. Dotted line is 1:1 correspondence between estimates.

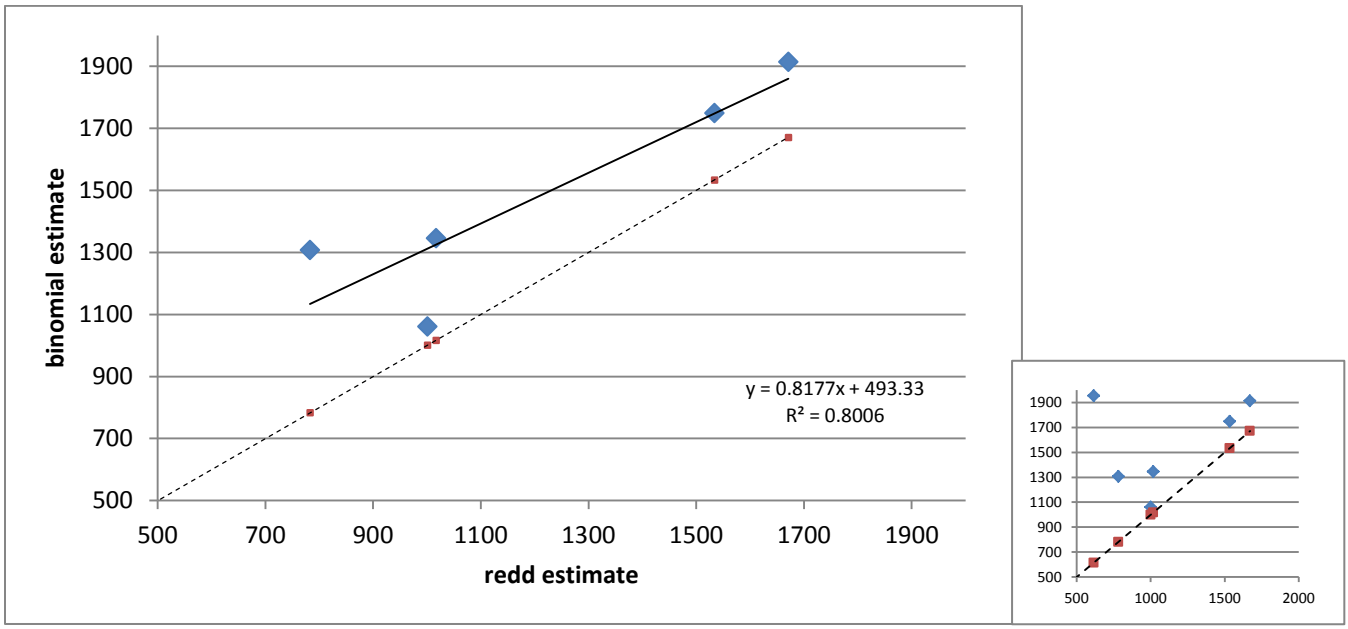
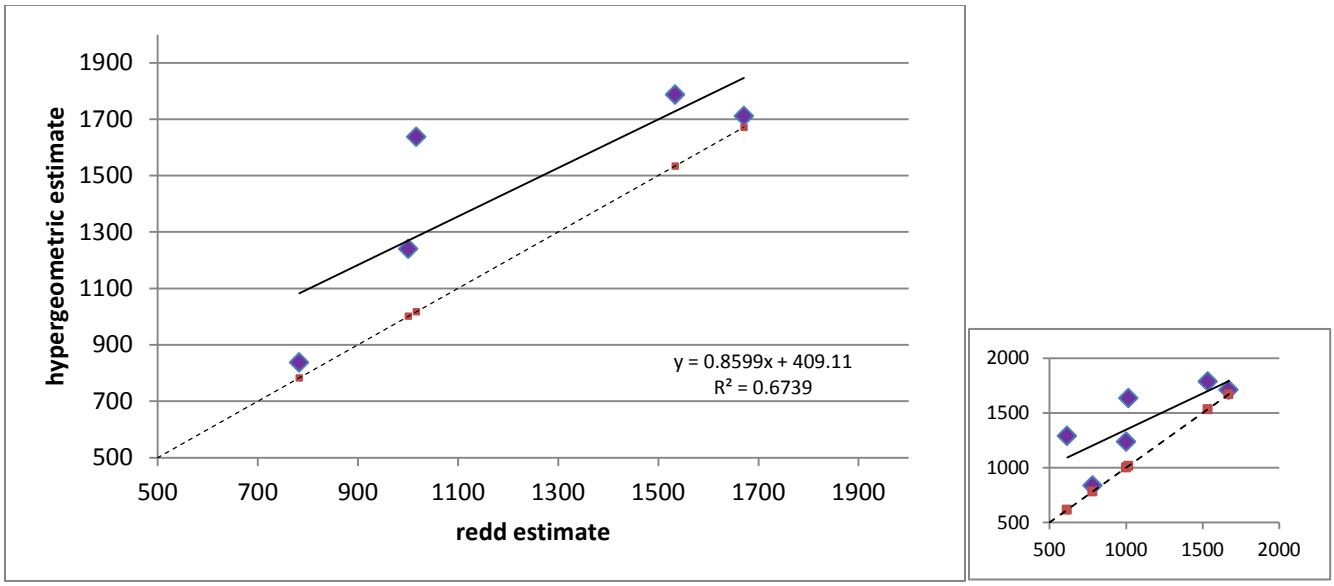


Figure 6. Comparison of hypergeometric tGMR estimates with redd-based estimates. Inset to right includes data from 2007 brood year. Dotted line is 1:1 correspondence between estimates.



Appendix 1. Juvenile data per week (Stat Wk) for each brood year, including estimated number of natural-born (wild) and hatchery-born juveniles, number of juveniles sampled for genetic analysis, number of putative yearlings and unmarked hatchery-born juveniles (based on assignment to hatchery broodstock), the total number of juveniles for tGMR, total captured genotypes (2 for each juvenile) included in the tGMR each week, and the total number of recaptures per week (recaptured genotypes).

Brood year 2007

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery-born Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parental assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parents assignments)
6	02/10/08	02/16/08	306	0	3			3	6	
7	02/17/08	02/23/08	241	0	1	1				
8	02/24/08	03/01/08	3078	0	6			6	12	
9	03/02/08	03/08/08	2079	0	11			11	22	1
10	03/09/08	03/15/08	5060	0	18			18	36	1
11	03/16/08	03/22/08	8130	0	14			14	28	
12	03/23/08	03/29/08	3157	0	11	1		10	20	1
13	03/30/08	04/05/08	5826	0	11			11	22	
14	04/06/08	04/12/08	3012	0	11			11	22	
15	04/13/08	04/19/08	6469	0	34			34	68	
16	04/20/08	04/26/08	4555	582	34			34	68	1
17	04/27/08	05/03/08	11848	22531	18	2	3	13	26	
18	05/04/08	05/10/08	22937	29430	71		2	69	138	
19	05/11/08	05/17/08	21764	53709	44	2	2	40	80	1
20	05/18/08	05/24/08	13838	26151	14		1	13	26	
21	05/25/08	05/31/08	18871	28084	19			19	38	1
22	06/01/08	06/07/08	20495	50872	90		4	86	172	5
23	06/08/08	06/14/08	11276	18269	49		1	48	96	2
24	06/15/08	06/21/08	11393	22848	20			20	40	1
25	06/22/08	06/28/08	5923	9283	8		1	7	14	
26	06/29/08	07/05/08	5856	15260	21			21	42	2
sum			186115	277019	508	6	14	488	976	16

Appendix 1 continued.

Brood year 2008

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parental assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parents assignments)
7	02/08/09	02/14/09	0	0						
8	02/15/09	02/21/09	0	0						
9	02/22/09	02/28/09	1044	0	10	1		9	18	
10	03/01/09	03/07/09	1334	0	27			27	54	2
11	03/08/09	03/14/09	3515	0	9			9	18	
12	03/15/09	03/21/09	1238	0	33			33	66	2
13	03/22/09	03/28/09	732	0	16		1	15	30	1
14	03/29/09	04/04/09	1215	0	33			33	66	1
15	04/05/09	04/11/09	3245	0	33	1	1	31	62	1
16	04/12/09	04/18/09	5206	0	49	3		46	92	1
17	04/19/09	04/25/09	8863	1251	119		1	118	236	10
18	04/26/09	05/02/09	5697	6729	34		1	33	66	5
19	05/03/09	05/09/09	14287	29720	124		1	123	246	12
20	05/10/09	05/16/09	11932	18132	185		2	183	366	17
21	05/17/09	05/23/09	14019	36009	73		1	72	144	3
22	05/24/09	05/30/09	10055	12338	0					
23	05/31/09	06/06/09	2702	2645	5			5	10	
24	06/07/09	06/13/09	4777	1821	5			5	10	
25	06/14/09	06/20/09	3013	0	2			2	4	
	sum		92871	108645	757	5	8	744	1488	55

Appendix 1 continued.

Brood year 2009

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parental assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parents assignments)
7	02/07/10	02/13/10	1196	0	24			24	48	11
8	02/14/10	02/20/10	6362	0	52	1	1	50	100	14
9	02/21/10	02/27/10	3578	0	2			2	4	
10	02/28/10	03/06/10	1671	0	3	1		2	4	1
11	03/07/10	03/13/10	8906	0	8			8	16	4
12	03/14/10	03/20/10	2066	0	7			7	14	1
13	03/21/10	03/27/10	2378	0	20	2		18	36	4
14	03/28/10	04/03/10	14408	0	106		3	103	206	23
15	04/04/10	04/10/10	21456	0	148	1	4	143	286	34
16	04/11/10	04/17/10	11309	17519	44			44	88	15
17	04/18/10	04/24/10	24446	10740	101			101	202	29
18	04/25/10	05/01/10	8833	15437	91		7	84	168	18
19	05/02/10	05/08/10	80339	87289	113		3	110	220	29
20	05/09/10	05/15/10	18346	13976	13			13	26	7
21	05/16/10	05/22/10	21326	34801	91		3	88	176	29
22	05/23/10	05/29/10	13338	7514	56		1	55	110	12
23	05/30/10	06/05/10	34276	21490	133		2	131	262	33
24	06/06/10	06/12/10	16718	16372	190		9	181	362	56
25	06/13/10	06/19/10	8153	4983	28			28	56	8
26	06/20/10	06/26/10	6680	3136	2			2	4	2
sum			305784	233258	1232	5	33	1194	2388	330

Appendix 1 continued.

Brood year 2010

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parental assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parents assignments)
7	02/14/11	02/20/11	612	0	15			15	30	
8	02/21/11	02/27/11	1806	0	8			8	16	
9	02/28/11	03/06/11	1024	0	6			6	12	1
10	03/07/11	03/13/11	1037	0	2			2	4	1
11	03/14/11	03/20/11	1378	0	26		1	25	50	2
12	03/21/11	03/27/11	718	0	9		1	8	16	1
13	03/28/11	04/03/11	989	114	37		1	36	72	2
14	04/04/11	04/10/11	1668	1889	63		9	54	108	6
15	04/11/11	04/17/11	528	1384	36		1	35	70	2
16	04/18/11	04/24/11	1688	10056	35		2	33	66	2
17	04/25/11	05/01/11	2764	16465	37		3	34	68	3
18	05/02/11	05/08/11	3284	21126	66		3	63	126	8
19	05/09/11	05/15/11	2047	9396	57		1	56	112	3
20	05/16/11	05/22/11	2089	13448	49			49	98	5
21	05/23/11	05/29/11	1988	14565	42		1	41	82	1
22	05/30/11	06/05/11	1490	9273	15		2	13	26	
23	06/06/11	06/12/11	559	3165	22		1	21	42	2
24	06/13/11	06/19/11	1091	10840	18		3	15	30	1
25	06/20/11	06/26/11	254	1774	1			1	2	
sum			27013	113496	544	0	29	515	1030	40

Appendix 1 continued.

Brood year 2011

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parental assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parents
6	2/5/2012	2/11/2012	488	0	9			4	8	1
7	2/12/2012	2/18/2012	1,644	0	45		1	15	30	4
8	2/19/2012	2/25/2012	7,560	0	187		1	90	180	24
9	2/26/2012	3/3/2012	7,387	0	154		1	68	136	17
10	3/4/2012	3/10/2012	5,481	0	155			75	150	16
11	3/11/2012	3/17/2012	4,842	0	135			60	120	13
12	3/18/2012	3/24/2012	3,577	0	69			27	54	7
13	3/25/2012	3/31/2012	8,778	0	136			58	116	13
14	4/1/2012	4/7/2012	6,951	0	117		2	52	104	12
15	4/8/2012	4/14/2012	10,375	618	141			64	128	8
16	4/15/2012	4/21/2012	34,542	16,491	140		1	65	130	9
17	4/22/2012	4/28/2012	14,924	21,150	68	1	2	28	56	4
18	4/29/2012	5/5/2012	23,957	32,445	324		8	267	534	45
19	5/6/2012	5/12/2012	8,275	9,023	90			44	88	10
20	5/13/2012	5/19/2012	16,073	4,136	151		2	69	138	9
21	5/20/2012	5/26/2012	40,854	59,587	304		1	138	276	17
22	5/27/2012	6/2/2012	12,009	33,552	270		3	113	226	9
23	6/3/2012	6/9/2012	8,032	8,291	162		3	105	210	12
24	6/10/2012	6/16/2012	3,840	2,862	31			11	22	1
25	6/17/2012	6/23/2012	6,559	3,527	145			62	124	10
26	6/24/2012	6/30/2012	4,985	8,354	10			16	32	1
27	7/1/2012	7/7/2012	766	1,548	8			4	8	0
sum			231,901	201,585	2851	1	25	1435	2870	242

Appendix 1 continued.

Brood year 2012

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parental assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parents assignments)
6	2/11/2013	2/17/2013	2,181	0	36			33	66	7
7	2/18/2013	2/24/2013	7,943	0	130			124	248	21
8	2/25/2013	3/3/2013	18,639	0	86			74	148	13
9	3/4/2013	3/11/2013	12,415	0	94			70	140	18
10	3/12/2013	3/19/2013	10,201	0	5			4	8	3
11	3/20/2013	3/27/2013	9,264	0	154			114	228	27
12	3/28/2013	4/4/2013	11,082	33,296	89			68	136	12
13	4/5/2013	4/12/2013	21,000	29,295	69	1	1	52	104	17
14	4/13/2013	4/20/2013	12,481	27,724	90	8		62	124	14
15	4/21/2013	4/28/2013	6,677	11,334	109	1		82	164	12
16	4/29/2013	5/6/2013	5,807	20,960	91			69	138	11
17	5/7/2013	5/14/2013	3,650	7,880	31			24	48	2
18	5/15/2013	5/22/2013	8,780	15,927	93		1	73	146	14
19	5/23/2013	5/30/2013	4,494	3,627	38			28	56	4
20	5/31/2013	6/7/2013	1,376	1,944	46			36	72	4
21	6/8/2013	6/15/2013	6,147	4,750	95			73	146	13
22	6/16/2013	6/23/2013	1,745	502	20			15	30	3
23	6/24/2013	7/1/2013	2,924	373	7			6	12	1
24	7/2/2013	7/9/2013	8,780	742	50			38	76	2
25	7/10/2013	7/17/2013	4,667	628	84			64	128	9
sum			160,253	158,984	1,417	10	2	1,109	2,218	207