2018 Broodyear Report: Abundance estimates for Stillaguamish River Chinook salmon using trans-generational genetic mark recapture

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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 1112 in more recent years (1996-2008). 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 Southern Boundary Restoration and Enhancement Fund (Southern Fund, SF) of the CTC funded this study to estimate the Chinook salmon spawning escapement using a transgenerational genetic mark-recapture (tGMR) protocol. We have provided the tGMR abundance estimates to the CTC to supplement information from redd estimates for calculating escapement estimates. 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 2019 to their parents: hatchery- and wild-origin natural spawners collected in 2018. Hatchery-origin spawners were more abundant and because no wild-origin females spawners were collected, only wild-origin male spawners were used to calculate difference in production between the spawner types (not significant). Using Chapman's (1951) approximation to the hypergeometric estimator, based on sampling without replacement, (genotyped spawners = marks, unique number of parents estimated by COLONY = captures, unique number of spawners assigning to juveniles = recaptures) we calculated spawner abundance for brood year 2018 and compared this abundance estimate to tGMR estimates calculated for brood years 2008-2017, and compared tGMR estimates to estimates derived from redd count expansions (Table 3). In all years except 2011, the confidence interval for tGMR estimates (hypergeometric or binomial) encompassed the redd-based estimate. The coefficient of variation based on Chapman's hypergeometric model met the CTC standard of 15% in six out of eleven broodyears.

Unmarked hatchery juveniles and yearling Stillaguamish Chinook salmon challenged the tGMR study design: if unaccounted for, unmarked hatchery juveniles and yearlings inflated abundance estimates because they increased capture numbers yet had no possible parents in the "Mark" pool. We screened for unmarked hatchery juveniles by assigning smolts to the hatchery brood stock and removed them prior to tGMR analyses (none suspected in 2019 juveniles). We identified four yearlings in 2019 by plotting smolt lengths and capture dates and observed (then removed) outliers smolts that were much longer that average smolt lengths in the time strata.

In this final document we summarize data from all project years covering brood years 2007-2018.

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 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 river also supports a smaller fall Chinook salmon population that transitioned in 2010 to an integrated stock, following the model of the summer-run program. The fall program includes captive brood collected from the South Fork Stillaguamish River, 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 number of breeders (N_b) in the Stillaguamish River upstream of the smolt trap site (RM 6) for brood year 2017 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 and contemporary redd-based escapement estimates. 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 is estimated only for spawners from brood years 2011 - 2018. We exclude the 2007 broodyear data because the redd count was estimated differently with Area Under the Curve, the sample sizes were small, and the weather was extreme that year, such that the coefficient of variation (CV) was nearly 30%. 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). Climate change has altered 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, reducing egg to migrant survival for Chinook in particular (Beamer and Pess 1999, Hall et al. 2014).

In response to changes in habitat and fisheries management approaches, Chinook salmon returns to the Stillaguamish River today are much reduced from the escapements documented in the late19th century. Estimates for historic Chinook salmon returns ranged from 9,700 - 13,321 per year as compared to an average of 1,081 in the past 20 years (1999-2018), based on redd surveys. 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 identifiable native stocks of Chinook salmon recognized in the Stillaguamish basin (Figure 1). The more abundant is a summer-run stock, 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). Because of low returns, the Tribe has not had a directed Chinook salmon fishery in over 25 years.

The second Chinook salmon stock on the Stillaguamish is a fall-run stock, which has declined precipitously; the average run is 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 as 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 2018 and compared estimates to brood years 2007 through 2017. The project used a genetic mark-recapture (GMR) protocol developed by Rawding et al. (2014) to estimate spawner abundances, employing a pooled Peterson estimate (Seber 1982) based on sampling with replacement, as well as Chapman's (1951) approximation to the hypergeometric estimator, based on sampling without replacement. 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, that marked and unmarked individuals have equal probability of capture, that marked individuals are correctly identified and their behavior is unaltered, and that 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. With the hypergeometric estimator, the out-migrating juveniles are assigned to parents using the program COLONY (described below), which estimates all parents, sampled and unsampled, giving rise to the juvenile sample. The unique number of estimated parents, sampled and unsampled, are the "captures", and the unique number of sampled parents assigning to juveniles are the "recaptures".

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, spawning surveys are challenging in the SF because of poor visibility and high flows.

Smolt samples are normally collected from February to July throughout their emigration period. Smolts were collected 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 six 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.

Collections and genotyping

We genotyped Chinook salmon spawner carcass samples collected in 2018 (see Table 1 for all collections throughout project) and genotyped smolts sampled from a mainstem smolt trap in 2019. We also genotyped tissue samples collected from the 2018 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 spawner carcasses 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). 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 GENEPOP (Rousset 2008) 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 GENEPOP). 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 with information about population conditions. Linkage disequilibrium provides evidence about 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 transgenerational genetic mark-recapture (tGMR) 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 hypergeometric estimator that used only unique recaptures, and a binomial estimator that allowed all recapture data regardless of whether the spawner had been previously recaptured in a sibling juvenile (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 program COLONY (Wang 2004, 2007, Wang and Santure 2009). 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 parent(s) for sibling families. 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 timely results and consistency. Initial runs included hatchery broodstock in the parent pool in order to identify unmarked hatchery juveniles. If any juveniles assigned to two hatchery broodstock parents, they were excluded and in subsequent runs only spawners collected on spawning grounds were included for parents. We ran the COLONY analyses multiple times and compared results. The final run was long length with full-pair likelihood and medium precision.

We used the COLONY 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):

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

We also used COLONY results to estimate spawners based on Chapman's (1951) approximation to the hypergeometric estimator. The hypergeometric is based on sampling without replacement and thus uses only unique parent assignments (no juveniles with a 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 (sampled and unsampled) and number of unique assignments to sampled parents (*M* = genotyped carcasses, *C* = unique number of parents, and *R* = unique assignments):

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

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

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

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 2019, 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 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. After reviewing data from several years and multiple studies, we concluded that the hypergeometric method is less sensitive to variation in family size and appears to better calculate abundance.

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:

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

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:

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

The proportion of natural origin spawners (pNOS) = 1 – pHOS and the var(pNOS) = var(pHOS). The number of hatchery origin spawners (HOS) was estimated by:

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

where N is the escapement estimate.

The number of natural origin spawners (NOS) was estimated by:

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

The variance for HOS was estimated by:

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

and the variance for NOS was estimated by:

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

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

Genotyping success was high for carcasses, smolts and the hatchery broodstock. In weeks 11-14, smolts from another species (likely Chum salmon) were inadvertently collected in the smolt trap, so numbers of genotyped smolts for those weeks were slightly lower than anticipated (see Appendix I). Only 10/64 spawners collected in 2018 were wild-born, which was too few for calculating some statistics (Table 4). Genetic statistics indicated that the carcass collections were random samples (Table 4). Genetic statistics also indicated that juvenile collections included families: linkage disequilibrium was higher than expected by chance after correcting for multiple tests.

Identifying unmarked hatchery juveniles and yearlings

The regression plot suggested four yearlings were mixed in with sub-yearlings (Figure 4). These juveniles were removed from the analysis.

Genetic Mark Recapture

Spawner estimates

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. (Table 3 and 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 estimates to be less than tGMR estimates. In each year (Figure 3), the point estimates and the upper bounds from the rarefaction curves were below the hypergeometric estimates (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 83.3% (95%CI = 73.3% - 93.3%) and pNOS estimate was 16.7% (95%CI = 6.6% - 26.7%). Using the hypergeometric distribution, the estimate for HOS was 805 fish (95%CI = 501 - 1108) and the estimate for NOS was 161 fish (95%CI = 0 - 327).

Parent recaptures indicated that the hatchery-origin spawners produced more offspring than natural-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 natural-origin spawners was not significant in 2018 or in any other year. For the 2018 spawners, reproductive success was uncorrelated with size. With the exception of the 2014 brood year, in 5/8 years of the study where the brood year collection was larger than 100 spawners, reproductive success was correlated with size (Table 3). Reproductive success was also positively correlated with sex: on average, females had more offspring than males, and in two out of eight years the difference was significant.

Estimate redd count expansion factor

We have a total of 11 years of tGMR and redd-based estimates (Figure 5). Graphical analysis of the data showed that the redd-based estimates were lower than the binomial and hypergeometric estimates. 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. The redd-based estimates explained 65% of the variance in the binomial estimates and explained 83% of the variance in the hypergeometric estimates. Dan Rawding (WDFW) conducted an analysis of correlations between redd-based escapement estimates and survey conditions (survey frequency, percentage of basin surveyed, environmental conditions), and mark-recapture estimates. The analysis suggested that survey covariates were uninfluential in the relationship between redd- and genetic-based estimates. Kris Ryding (WDFW) used historical abundance data and tGMR data to adjust historical fall Chinook spawner estimates in two steps. Briefly, 1) convert historical flight based escapement estimates to equivalent ground based escapement estimates using the ratio between the estimates from years where flight and ground methods were conducted in parallel, 2) convert all ground based (redd) escapement estimates to GMR estimates using the regression of the GMR estimates on redd based estimates from years where ground and tGMR methods were conducted in parallel.

Discussion

Chinook salmon spawner abundance is a key parameter for 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 turbid and some spawning areas are inaccessible to biologists. 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 (Seber 1982). 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* with the direction and magnitude of the bias determined by the violation. In this study, we use individual spawner genotypes as the "mark" and use spawner genotypes assigning to juveniles 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 estimated *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 identified incorrectly as offspring of unsampled parents. This would bias estimated *N* 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 identified incorrectly 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 estimated *N* higher than the true value because we would not assign juveniles to their sampled parent. If our mismatch criterion is too relaxed, it would bias estimated *N* 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 storms prevent equal sampling over the spawning period. Further, in some years the offspring per spawner and carcass recoveries favored males and in other years favored females. 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. 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, spawner length may consistently 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, in all but the 2013 brood year 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.

Another possible source of bias could arise from sampling only unmarked, presumably natural-origin spawners for brood years 2008 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- 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 2011-2018.

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. In 2011, 2014 and 2018 hatchery-origin spawners were more abundant and in 2012, 2013, 2015, 2016, and 2017 natural-origin spawners were more abundant, but in all years with data on hatchery- and natural-origin spawners there was no significant difference in offspring production between the two groups. 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 years.

Comparison among techniques

For each brood year, the binomial and hypergeometric estimates were similar (Figure 2). 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 susceptible to bias when recoveries are low 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 but 2013, the CI's overlapped between the binomial and hypergeometric estimates and the redd-based estimate mostly aligned with either or both tGMR 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 estimates were 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 in years where high water challenges redd counting, which likely leads to an underestimate in the redd count. Further, sustained high flows flush carcasses from the system making carcass recovery difficult, resulting in fewer genetic “marks” for high water years. 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 16%, which just exceeded 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 closer in 2012. In 2014 and 2015 weather anomalies led to no snow pack and early snow melt, respectively. In both years few spawners were able to enter the river and few outmigrating juveniles were captured. The tGMR estimate in 2014 was essentially the same as the redd-based estimate. The 2015 broodyear had the fewest juveniles captured and fewest marks recaptured (Table 3), the confidence interval for the tGMR hypergeometric estimate encompassed the redd-based estimate and the CV of the tGMR estimate (26%) was the highest for all years with full basin survey (2008 onward). In 2016 a modest number of carcasses were recovered but the smolt sample was good and the CV for the tGMR hypergeometric estimate was 17%. In 2017 the carcass sample was abundant and relatively few juveniles were captured, but the CV was within the goal of 15%. In 2018, the same number of carcasses were collected as in 2016, but fewer juveniles were captured. Similar to 2016, the hypergeometric confidence interval overlapped with the redd-based estimate and the CV for the hypergeometric was 17.6%. Among all years, sampling was best for the 2013 brood year with a high number of marks, captures and recaptures. The tGMR estimate for 2018 (966 spawners) suggested that ~ 7% of the estimated number of spawners had been sampled (64 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 (wild plus hatchery) are captured in the smolt trap. Because of abundant hatchery food and warmer water temperatures, hatchery juveniles tend to be larger at release than are natural-origin juveniles of the same age. However, hatchery and wild juvenile size distributions overlap, so we were unable to identify hatchery-origin juveniles just by size and relied instead on assignments to hatchery broodstocks to identify unmarked hatchery juveniles. However, previous results suggested that unmarked hatchery juveniles had little impact on tGMR abundance estimates (Small et al. 2012, 2013). 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 seven of eleven years for the binomial model and in four of eleven years for the hypergeometric model. Given that juvenile sampling efforts are standardized (although weather can interfere with juvenile sampling), we could increase adult tissue sample collections to better meet precision standards. The CTC standards for unbiased estimates were largely achieved, although 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, improvement in sample quality (freshness) has increased the number of carcass genotypes and 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 2018 brood year study are in bold type.

WDFW code	Collection Location and run type (if known)	Life stage	N	N >9 loci	hatchery	yearling
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		
13BF	North Fork Stillaguamish summer (spawning grounds, wild and hatchery)	adult	231	211		
14DN	North Fork Stillaguamish summer (spawning grounds, wild and hatchery)	adult	62	61		
15BG	North Fork Stillaguamish summer (spawning grounds, wild and hatchery)	adult	52	49		
16DO	North Fork Stillaguamish summer (spawning grounds, wild and hatchery)	adult	69	63		
17DD	North Fork Stillaguamish summer (spawning grounds, wild and hatchery)	adult	214	195		
18CE	**North Fork Stillaguamish summer (spawning grounds, wild and hatchery)**	**adult**	**68**	**64**		
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		
13BI	North Fork Stillaguamish summer (hatchery broodstock)	adult	112	112		
14DP	North Fork Stillaguamish summer (hatchery broodstock)	adult	133	133		
15BE	North Fork Stillaguamish summer (hatchery broodstock)	adult	119	119		
16DL	North Fork Stillaguamish summer (hatchery broodstock)	adult	135	135		
17DE	North Fork Stillaguamish summer (hatchery broodstock)	adult	128	128		
18CA	North Fork Stillaguamish summer (hatchery broodstock)	adult	131	131		
19DL	**North Fork Stillaguamish summer (hatchery broodstock)**	**adult**	131	131		
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
14DM	Mainstem Stillaguamish (subsampled from 1597 juveniles)	smolt	1169	1148	5	2
15BF	Mainstem Stillaguamish (trap pulled beginning of June)	smolt	224	181(43*)	3	3
16DN	Mainstem Stillaguamish (trap pulled end of June)	smolt	98	92	1	0
17DC	Mainstem Stillaguamish (subsampled from 1254 juveniles)	smolt	893	879	0	1
18CD	Mainstem Stillaguamish	smolt	617	474	0	1
19DJ	**Mainstem Stillaguamish**	**smolt**	**446**	**432***	0	4

*14 smolts were either not Chinook salmon or were contaminated

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 2008-2018 using transgenerational genetic mark-recapture (tGMR) based on the hypergeometric model. Genotyped carcasses are under "Marks", genotyped outmigrating juveniles are the data for COLONY to estimate the unique number (N) of parents giving rise to the juveniles (Captures), juveniles assigned to a unique spawner are under "Recaptures" (sampling without replacement), and the coefficient of variation for the tGMR estimate is under "CV". Redd-based abundance estimates were from expanded redd counts. The tGMR estimates are from the hypergeometric estimate and excluded unmarked hatchery juveniles and yearlings.

Brood Year	Genotyped carcasses (Marks)	Genotyped juveniles	estimated N unique parents (Captures)	N unique recaptures of parents (Recaptures)	tGMR Estimate hypergeometric	tGMR CV	Redd-based estimate
2008	72	744	468	19	1711	18%	1671
2009	147	1194	703	83	1239	7%	1001
2010	54	515	334	21	837	16%	783
2011	112	1435	854	58	1637	6%	1017
2012	164	1109	855	78	1787	8%	1534
2013	211	1141	404	85	997	9%	854
2014	61	171	121	17	419	18%	432
2015	49	126	91	9	709	26%	459
2016	63	879	345	20	1053	17%	861
2017	194	473	301	54	1070	10%	1075
2018	64	428	333	18	966	18%	665

Table 4. Genetic statistics include gene diversity (GeneDiv, expected heterozygosity corrected for collection size), allelic richness (A_R, 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. The number of linked pairs of loci were calculated at $p < 5\%$ and $< 1\%$). Both hatchery- and wild-origin spawners were collected in the North Fork Stillaguamish River spawners in 2011-2019, and statistics were calculated for the spawners together and for the spawners grouped by origin. The wild-origin spawners collected in 2018 were too few to calculate some of the genetic statistics.

					deficit	excess	linkage (91 pairs)	
	N	GeneDiv	A_R	F_{IS}	*p* value	*p* value	5%	1%
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	0.028	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	0.001	0.999	8	3
13NF spawners	211	0.8716	14.24	0.001	0.426	0.575	11	1
13NF_h_spawners	102	0.8687	13.94	-0.001	0.528	0.473	18	6
13NF_w_spawners	107	0.8726	14.45	0.002	0.419	0.582	12	2
14NF spawners	61	0.8791	13.55	-0.002	0.560	0.440	4	1
14NF_h_spawners	36	0.8741	14.02	0.008	0.322	0.679	1	0
14NF_w_spawners	25	0.8784	13.86	0.003	0.381	0.620	5	1
15NF spawners	49	0.8717	14.39	0.026	0.023	0.977	2	0
16NF_spawners	63	0.8739	14.32	0.039	0.000	1.000	4	1
16NF_h_spawners	22	0.8728	14.22	0.030	0.020	0.980	1	1
16NF_w_spawners	41	0.8753	13.98	0.053	0.002	0.998	7	3
17NF_spawners	193	0.8691	14.20	0.004	0.271	0.730	8	3
17NF_h_spawners	86	0.8670	12.13	-0.004	0.665	0.337	17	6
17NF_w_spawners	107	0.8708	13.42	0.010	0.120	0.881	8	2
18NF_spawners	64	0.8680	16.77	0.000	0.511	0.490	3	2
18NF_h_spawners	54	0.8634	16.38	-0.007	0.684	0.316	3	1
18NF_w_spawners	10	0.8908	NA	0.029	0.209	0.867	NA	NA
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
13NF broodstock	112	0.8755	13.30	0.011	0.117	0.884	8	3
14NF broodstock	133	0.8736	13.99	0.008	0.153	0.847	4	2

					deficit	excess	linkage (91 pairs)	
	N	GeneDiv	A_R	F_{IS}	*p* value	*p* value	5%	1%
15NF broodstock	119	0.8708	14.07	-0.006	0.789	0.211	7	3
16NF broodstock	135	0.8708	14.29	-0.006	0.787	0.214	9	2
17NF broodstock	128	0.8530	12.90	0.009	0.131	0.869	15	7
18NF broodstock	131	0.8735	16.75	0.003	0.360	0.650	14	5
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
14 mainstem smolts	1141	0.8725	14.27	0.013	<u>0.000</u>	1.000	85	74
15 mainstem smolts	181	0.8731	13.67	0.006	0.197	0.803	43	27
16 mainstem smolts	91	0.8559	13.83	-0.007	0.736	0.266	23	8
17 mainstem smolts	879	0.8689	14.94	0.004	0.072	0.932	55	42
18 mainstem smolts	473	0.8705	14.66	-0.012	0.997	<u>0.003</u>	64	43
19 mainstem smolts	426	0.8709	16.76	0.009	0.0157	0.985	60	36

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

Brood year		binomial	hypergeometric	redd
2008	N	1914	1711	1671
	Var	61881	96936	
	CV	13%	18%	
2009	N	1061	1239	1001
	Var	2921	6888	
	CV	5%	7%	
2010	N	1358	837	783
	Var	39092	17076	
	CV	15%	16%	
2011	N	1345	1637	1017
	Var	6914	10465	
	CV	6%	6%	
2012	N	1750	1787	1534
	Var	13273	18903	
	CV	7%	8%	
2013	N	1469	997	854
	Var	5614	8939	
	CV	5%	9%	
2014	N	721	419	432
	Var	15884	5623	
	CV	17%	18%	
2015	N	598	709	459
	Var	20505	34080	
	CV	24%	26%	
2016	N	1759	1053	861
	Var	46613	31897	
	CV	12%	17%	
2017	N	1285	1070	1075
	Var	9731	12021	
	CV	8%	10%	
2018	N	1396	966	665
	Var	55086	28853	
	CV	17%	18%	

Table 6. Reproductive success by sex and by spawner group in upper portion of table and generalized linear model results in lower portion of table (for years where spawner status was recorded). 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,(2011by, df=111, residual df=107; 2012by df=163, residual df=159; 2013by df=227, residual df=223; 2014by, df=61, residual df=57; 2015by, df=8, residual df=4; 2016by, df=19, residual df=15; 2017by, df=193, residual df=189).

status		2011BY M	2011BY F	2012BY M	2012BY F	2013BY M	2013BY F
H	N_{adult}	21	44	28	40	35	52
	tot N_{off}	13	102	20	63	23	96
	avg N_{off}	0.62	2.32	0.71	1.58	0.66	1.84
W	N_{adult}	22	25	39	57	40	84
	tot N_{off}	27	96	42	82	47	161
	avg N_{off}	1.23	3.84	1.08	1.44	1.18	1.92

GLM	2011BY Estimate	Std. Error	z value	Pr(>|z|)	2012BY Estimate	Std. Error	z value	Pr(>|z|)	2013BY Estimate	Std. Error	z value	Pr(>|z|)
fork length	**0.0041**	0.0013	**3.096**	**0.002**	**0.0036**	0.0012	3.086	**0.002**	**0.0027**	0.0013	2.117	**0.034**
sex	**-0.7673**	0.3341	**-2.297**	**0.022**	-0.1879	0.2720	-0.691	0.490	**-0.6826**	0.3023	-2.258	**0.024**
Origin (H v W)	0.4794	0.2828	1.695	0.090	-0.0225	0.2459	-0.091	0.927	0.0908	0.2872	0.316	0.752
collection date	0.0174	0.0131	1.334	0.182	-0.0022	0.0114	-0.190	0.849	**0.0492**	0.0168	2.938	**0.003**

status		2014BY M	2014BY F	2015BY M	2015BY F	2016BY M	2016BY F
H	N_{adult}	19	17	6	7	12	10
	tot N_{off}	7	9	0	2	0	11
	avg N_{off}	0.37	0.53		0.29		1.10
W	N_{adult}	17	8	17	19	26	15
	tot N_{off}	4	8	4	8	23	28
	avg N_{off}	0.24	1	0.24	0.42	0.88	1.87

GLM	2014BY Estimate	Std. Error	z value	Pr(>|z|)	2015BY Estimate	Std. Error	z value	Pr(>|z|)	2016BY Estimate	Std. Error	z value	Pr(>|z|)
fork length	**0.0056**	0.0022	2.504	**0.012**	0.0066	0.0102	0.642	0.521	-0.0002	0.0014	-0.112	0.911
sex	-0.2474	0.5201	-0.476	0.634	0.8015	0.6077	1.319	0.187	-0.4482	0.3485	-1.286	0.198
Origin (H v W)	-0.1246	0.5181	-0.241	0.810	0.8535	0.8671	0.984	0.325	0.5351	0.4398	1.217	0.224
collection date	0.0391	0.0325	1.204	0.229	-0.0101	0.0665	-0.152	0.879	-0.0133	0.0106	-1.254	0.210

status		2017BY M	2017BY F	2018BY M	2018BY F
H	N_{adult}	52	34	30	15
	tot N_{off}	32	12	11	5
	avg N_{off}	0.62	0.35	0.37	0.33
W	N_{adult}	40	68	9	0
	tot N_{off}	28	70	2	0
	avg N_{off}	0.70	1.03	0.22	0

GLM	2017BY Estimate	Std. Error	z value	Pr(>|z|)	2018BY Estimate	Std. Error	z value	Pr(>|z|)
fork length	**0.0047**	0.0015	3.108	**0.002**	-0.0002	0.0014	-0.112	0.911
sex	-0.3325	0.3552	-0.936	0.349	-0.4482	0.3485	-1.286	0.198
Origin (H v W)	0.2171	0.3842	0.565	0.572	0.5351	0.4398	1.217	0.224
collection date	-0.0061	0.0168	-0.361	0.718	-0.0133	0.0106	-1.254	0.210

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

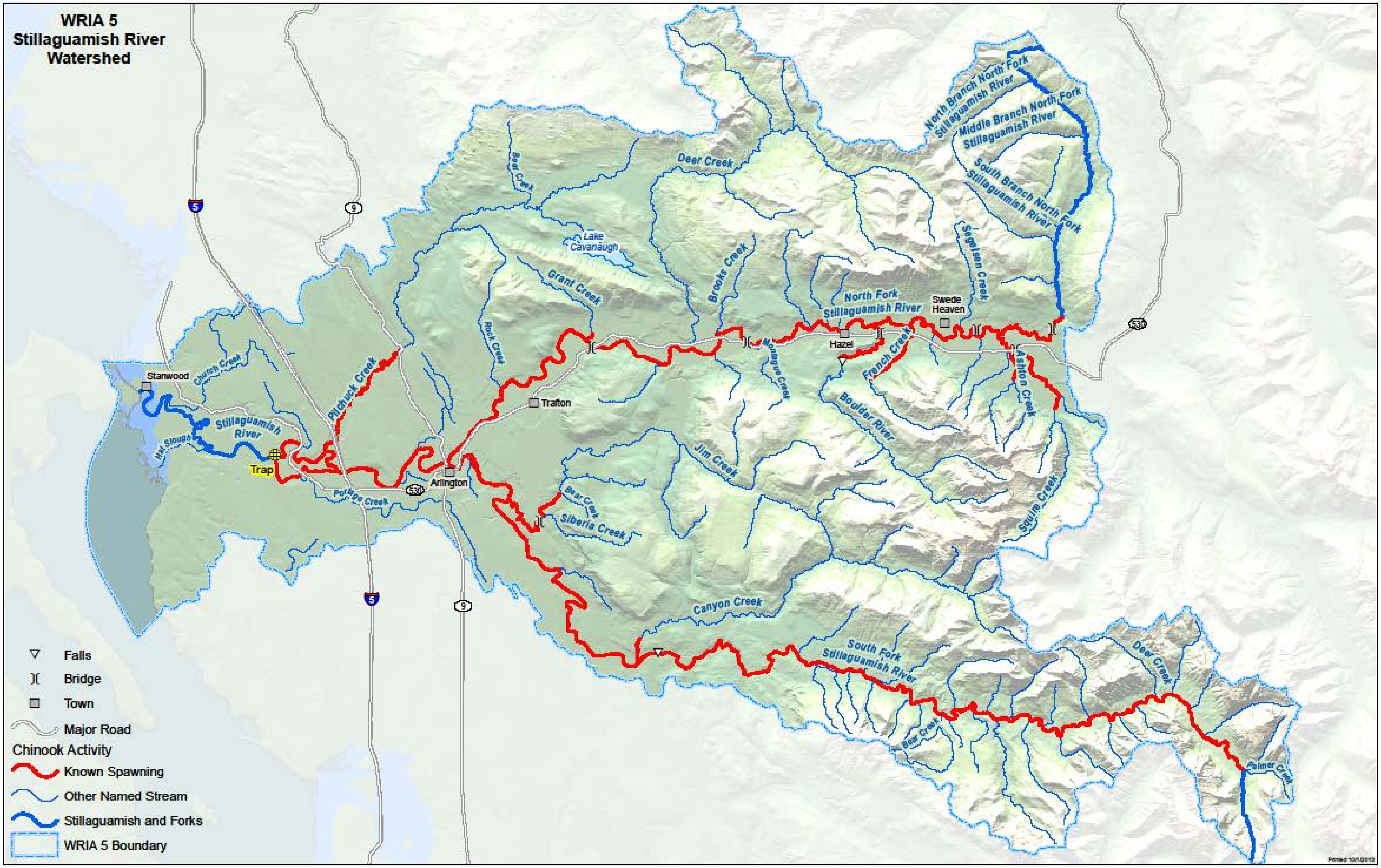


Figure 2. Comparison of the abundance estimates with different methods: binomial, hypergeometric, and redd-based estimate (redd).

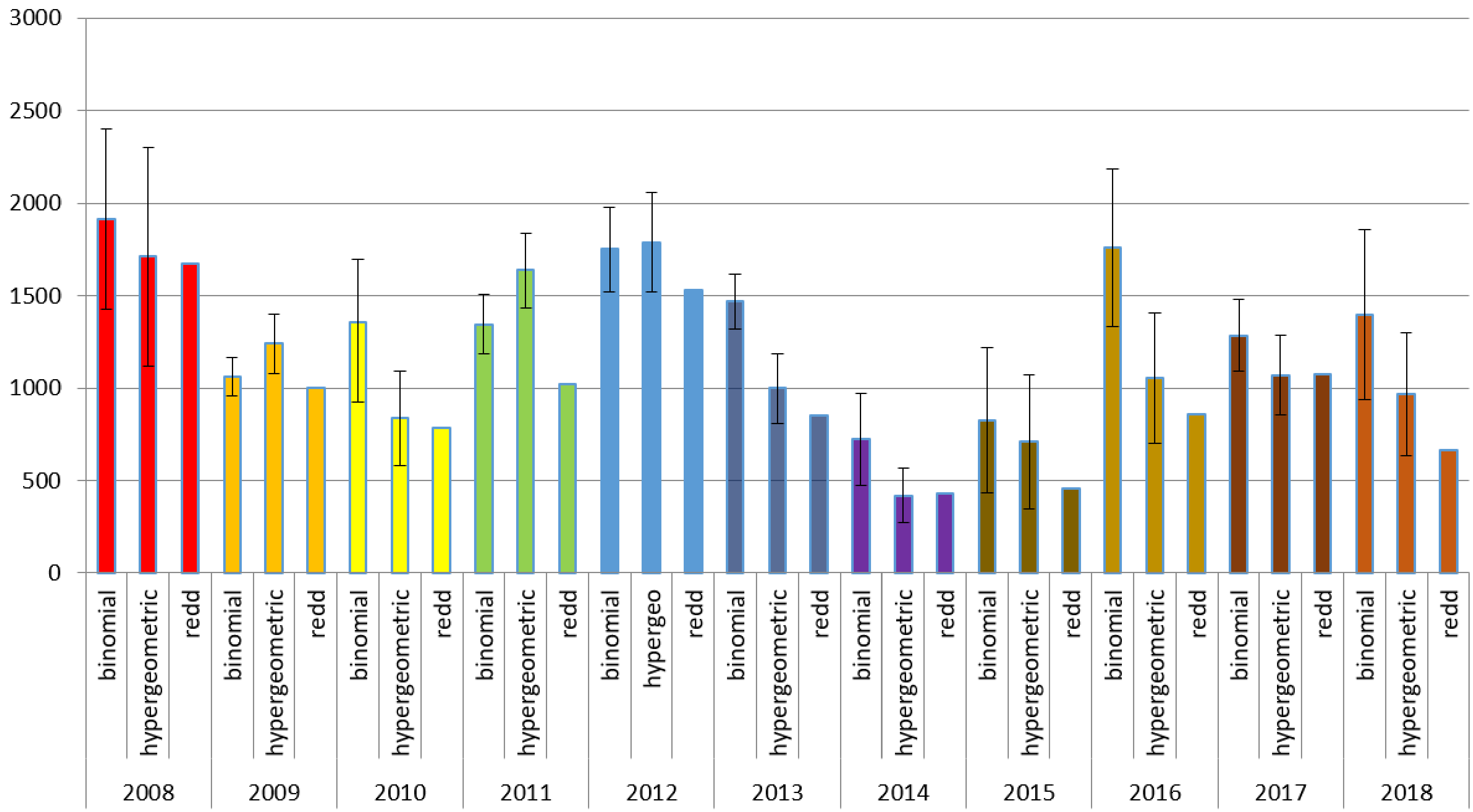


Figure 3. Rarefaction curves estimating spawners in brood years 2008 through 2018. The large dots are the averages over 10,000 re-samples.

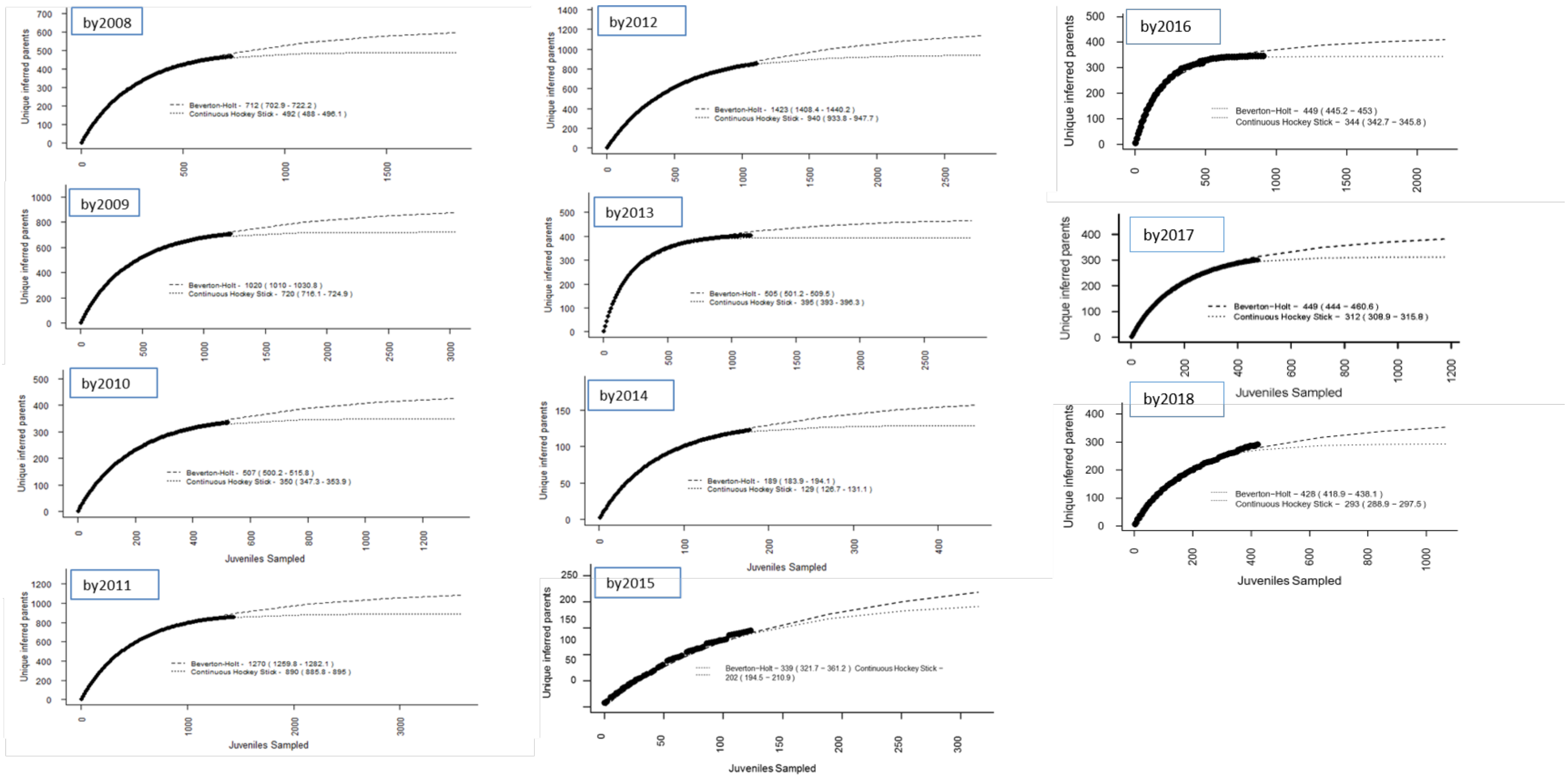


Figure 4. Plot of unclipped smolt lengths versus out-migration day in 2019. Putative yearlings are in red and were removed from data set before analyses.

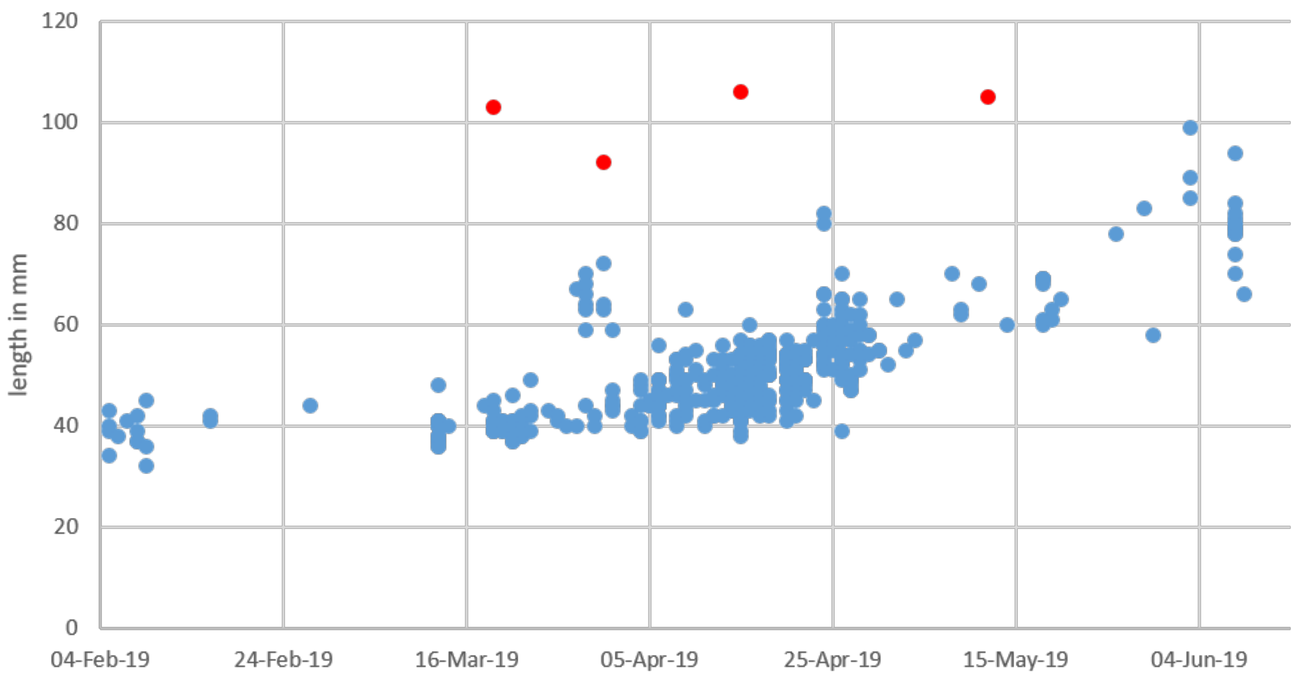
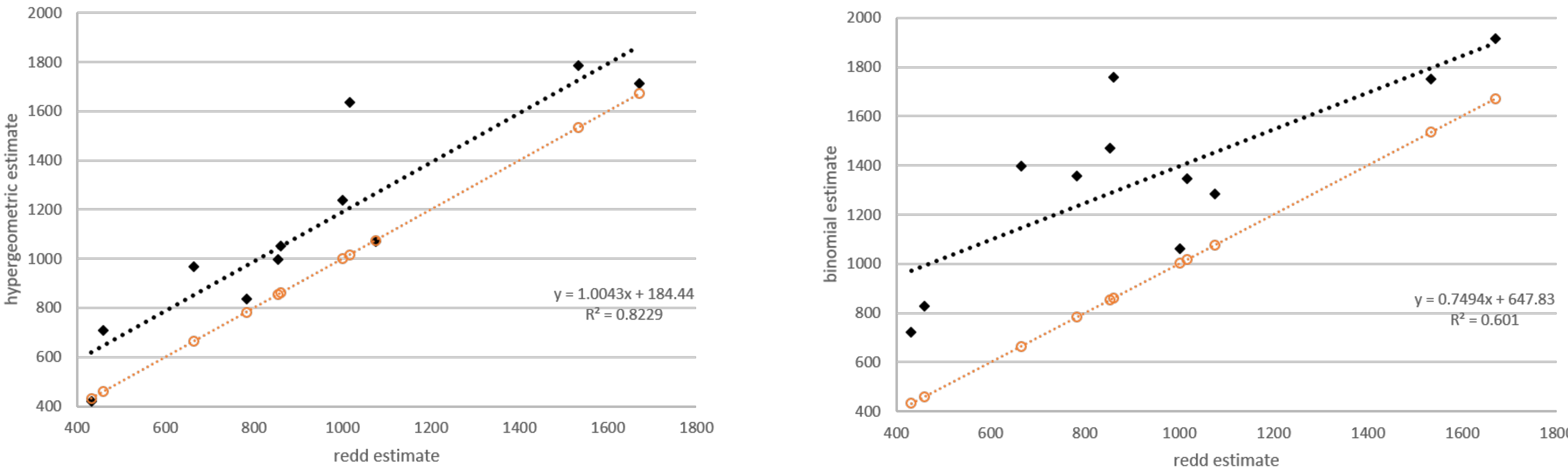


Figure 5. Comparison of hypergeomentric tGMR estimates with redd-based estimates (left) and comparison of binomial tGMR estimates with redd-based estimates (right). Dotted orange line with orange circles is 1:1 correspondence between estimates. Dashed blue line is the regression line for the data (blue diamonds).



Appendix 1. Juvenile data per week (Stat Wk) for each brood year in reverse chronological order, 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 2018

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent assignments)
6	2/3/2019	2/9/2019	20,085	0	12			11	22	1
7	2/10/2019	2/16/2019	2,431	0	2			2	4	
8	2/17/2019	2/23/2019	0	0					0	
9	2/24/2019	3/2/2019	313	0	1			1	2	
10	3/3/2019	3/9/2019	0	0					0	
11	3/10/2019	3/16/2019	2,267	0	15			13	26	
12	3/17/2019	3/23/2019	4,888	0	27	1		20	40	
13	3/24/2019	3/30/2019	7,231	0	14			13	26	1
14	3/31/2019	4/6/2019	14,305	587	42	1		40	80	3
15	4/7/2019	4/13/2019	15,514	1,494	49			49	98	3
16	4/14/2019	4/20/2019	23,842	7,992	128	1		124	248	12
17	4/21/2019	4/27/2019	19,066	48,072	89			88	176	6
18	4/28/2019	5/4/2019	6,876	14,344	16			14	28	3
19	5/5/2019	5/11/2019	5,845	7,147	4			4	8	1
20	5/12/2019	5/18/2019	6,955	9,100	7	1		6	12	1
21	5/19/2019	5/25/2019	808	895	3			2	4	
22	5/26/2019	6/1/2019	20,761	12,793	3			3	6	
23	6/2/2019	6/8/2019	44,358	63,063	16			16	32	1
sum			195,544	165,484	428	4	0	406	812	32

Appendix I continued

Brood year 2017

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent assignments)
9	2/25/2018	3/3/2018	250	0	12			11	22	3
10	3/4/2018	3/10/2018	1,706	0	4			4	8	1
11	3/11/2018	3/17/2018	1,078	0	26			18	36	5
12	3/18/2018	3/24/2018	1,663	0	40			16	32	2
13	3/25/2018	3/31/2018	4,009	0	62			14	28	2
14	4/1/2018	4/7/2018	3,256	0	69			27	54	10
15	4/8/2018	4/14/2018	4,314	8,417	32			29	58	9
16	4/15/2018	4/21/2018	4,305	13,218	51			49	98	11
17	4/22/2018	4/28/2018	7,298	10,368	33			33	66	11
18	4/29/2018	5/5/2018	7,925	12,216	80	1		79	158	29
19	5/6/2018	5/12/2018	7,464	28,219	46			45	90	12
20	5/13/2018	5/19/2018	8,611	72,337	80			75	150	21
21	5/20/2018	5/26/2018	4,640	4,346	42			41	82	12
22	5/27/2018	6/2/2018	0	2,513	8			8	16	3
23	6/3/2018	6/9/2018	4,927	23,644	1			1	2	1
24	6/10/2018	6/16/2018	3,711	4,606	25			23	46	10
25	6/17/2018	6/23/2018	0	1,632						
sum			65,159	181,518	611	1		473	946	142

Appendix I continued

Brood year 2016

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent assignments)
6	2/5/17	2/11/17	326	0	1			1	2	
7	2/12/17	2/18/17	2,167	0	17			9	18	
8	2/19/17	2/25/17	1,676	0	40			27	54	2
9	2/26/17	3/4/17	2,110	0	43			28	56	5
10	3/5/17	3/11/17	892	0	9			6	12	3
11	3/12/17	3/18/17	1,402	0				0	0	
12	3/19/17	3/25/17	2,657	0	56			32	64	3
13	3/26/17	4/1/17	3,068	0	54			36	72	3
14	4/2/17	4/8/17	3,514	0	86			56	112	3
15	4/9/17	4/15/17	3,719	756	90			55	110	3
16	4/16/17	4/22/17	5,314	20,154	132	1		95	190	8
17	4/23/17	4/29/17	23,003	56,205	111			83	166	5
18	4/30/17	5/6/17	9,908	28,869	112			82	164	3
19	5/7/17	5/13/17	14,198	12,216	122			89	178	11
20	5/14/17	5/20/17	14,603	22,304	164			120	240	4
21	5/21/17	5/27/17	6,044	11,332	76			56	112	7
22	5/28/17	6/3/17	8,675	14,894	74			56	112	
23	6/4/17	6/10/17	5,809	5,156	38			28	56	1
24	6/11/17	6/17/17	1,615	893	9			7	14	1
25	6/18/17	6/24/17	2,688	255	19			12	24	
26	6/25/17	7/1/17	573	0	1			1	2	
sum			113,961	173,034	1,254	1	0	879	1,758	62

Appendix 1 continued

Brood year 2015

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent assignments)
5	01/31/16	02/06/16	84		1			1	2	
6	02/07/16	02/13/16	33		1			1	2	1
7	02/14/16	02/20/16	185							
8	02/21/16	02/27/16	920		3			3	6	1
9	02/28/16	03/05/16	5,530		15			15	30	3
10	03/06/16	03/12/16	3,855		23			23	46	4
11	03/13/16	03/19/16	668		2			2	4	
12	03/20/16	03/26/16	417		2			2	4	
13	03/27/16	04/02/16	3,814		11			11	22	1
14	04/03/16	04/09/16	5,635		8		1	6	12	1
15	04/10/16	04/16/16	4,168	4,119	4			4	8	1
16	04/17/16	04/23/16	5,270	23,765	4			4	8	1
17	04/24/16	04/30/16	2,153	59,118	6			6	12	
18	05/01/16	05/07/16								
19	05/08/16	05/14/16		13,831						
20	05/15/16	05/21/16		65,775						
21	05/22/16	05/28/16		132						
22	05/29/16	06/04/16	877	1,636	1			1	2	
23	06/05/16	06/11/16	11,567	2,111	1			1	2	
24	06/12/16	06/18/16	2,014	10,545	9			9	18	1
25	06/19/16	06/25/16	448	448	2			2	4	
sum			47,639	181,480	93*		1	91*	182*	14

*smolt sample from mainstem trap was augmented with smolts sampled in the delta

Appendix 1 continued

Brood year 2014

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent assignments)
7	02/08/2015	02/14/2015	368	0	2			2	4	
8	02/15/2015	02/21/2015	2,292	0	2	1		1	2	
9	02/22/2015	02/28/2015	5,342	0	0(19)				0	
10	03/01/2015	03/07/2015	3,167	0	1(9)			1	2	
11	03/08/2015	03/14/2015	2,847	0	0(13)				0	
12	03/15/2015	03/21/2015	4,713	0	22(23)		1	21	42	3
13	03/22/2015	03/28/2015	2,882	0	24(25)			24	48	4
14	03/29/2015	04/04/2015	5,880	0	36	1		35	70	6
15	04/05/2015	04/11/2015	5,735	1,555	21(22)	1	2	18	36	5
16	04/12/2015	04/18/2015	5,476	50,137	30(31*)		3	27	55	2
17	04/19/2015	04/25/2015	6,480	53,609	30			30	60	6
18	04/26/2015	05/02/2015	2,180	44,023	10			10	20	2
19	05/03/2015	05/09/2015	170	88,29	1			1	2	
20	05/10/2015	05/16/2015	4,617	2,211	1			1	2	
21	05/17/2015	05/23/2015	0	6,906	0				0	
22	05/24/2015	05/30/2015	0	0	0				0	
23	05/31/2015	06/06/2015	0	0	0				0	
sum			52,149	167,270	223(180)	3	6	171	342	28

*one pair of samples had matching genotypes

Appendix 1 continued.

Brood year 2013

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent assignments)
7	2/10/2014	2/16/2014	2,176	0	60			58	116	21
8	2/17/2014	2/23/2014	3,169	0	76			65	130	30
9	2/24/2014	3/2/2014	3,480	0	50			45	90	21
10	3/3/2014	3/9/2014	3,742	0						
11	3/10/2014	3/16/2014	9,434	0	77	1		65(66)	132	11
12	3/17/2014	3/23/2014	74,481	0	83			76	152	24
13	3/24/2014	3/30/2014	5,189	0	100			97	194	23
14	3/31/2014	4/6/2014	2,828	0	106			102	204	19
15	4/7/2014	4/13/2014	2,101	0	150	1		139(140)	280	31
16	4/14/2014	4/20/2014	2,987	0	70			61	122	15
17	4/21/2014	4/27/2014	3,827	0	111		2	106(108)	216	37
18	4/28/2014	5/4/2014	10,248	1,099	133		1	130(131)	262	41
19	5/5/2014	5/11/2014	3,463	1,682	62			32	64	8
20	5/12/2014	5/18/2014	14,226	38,304	141			46	92	13
21	5/19/2014	5/25/2014	14,581	51,961	129		1	41(42)	84	11
22	5/26/2014	6/1/2014	20,379	12,364	118			39	78	15
23	6/2/2014	6/8/2014	15,879	9,424	42		1	12(13)	26	2
24	6/9/2014	6/15/2014	9,402	1,437	19			6	12	
25	6/16/2014	6/22/2014	24,199	4,174	54			17	34	4
26	6/23/2014	6/29/2014	3,502	1,459	12			4	8	1
sum			229,293	121,905	1,593	2	5	1,141(1,148)	2,296	327

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 parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parent 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

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 parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parent
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 2010

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parent 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 2009

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single or both parent 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 2008

Stat Wk	Start	End	Wild Outmigration Estimate	Hatchery Outmigration Estimate	Juveniles collected for DNA	Yearling juveniles based on length	Hatchery juveniles based on parent assignments	Juveniles for mark-recapture	Total Captured Genotypes	Recaptured Genotypes (includes single and both parent 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		92,871	108,645	757	5	8	744	1,488	55