2011 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 a study to estimate the Chinook salmon spawning escapement using a genetic mark-recapture (GMR) protocol. The GMR 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 years 2008-2011 to their parents (natural spawners collected years 2007-2010, each juvenile set was assigned to its respective potential parent group). Using a pooled Lincoln-Peterson estimate (genotyped spawners = marks, genotyped outmigrating juveniles = captures, juveniles assigned back to spawners = recaptures) we calculated spawner abundance in each of four years and compared these abundance estimates to estimates derived from redd count expansions (Table 1). In brood years 2007, 2008, and 2010 the GMR estimates were higher than the redd count estimate and in brood year 2009 the GMR and redd count estimates aligned.

Stillaguamish Chinook salmon juveniles presented challenges to the GMR 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 hatchery broodstocks (genetic assignment) for their respective brood years 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, both adjustments resulted in small changes in the GMR estimates. The GMR results presented here include corrections for unmarked hatchery juveniles and yearlings.

This GMR study was opportunistic in that it used genetic samples collected for another project that evaluated genetic differences between the North and South forks of the Stillaguamish River. Despite the opportunistic nature, the coefficient of variation (CV) based on Bailey's binomial model, was higher than the CTC standard of 15% in only one of four years. The Sentinel Stocks program provided $117,297 US for the Stillaguamish River Chinook salmon GMR project in 2011.

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 desirable 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 years 2007-2010 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. This 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 GMR project, only natural-origin spawners were sampled – abundance by origin will be estimated starting in brood year 2011 when both hatchery- and natural-origin spawners will be sampled in spawning areas. This project also employed previously collected data from smolt trapping conducted late January through June in years 2008, 2009, 2010 and smolt data collected for the GMR in 2011. 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 program fish and 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). The Tribe's smolt and spawner monitoring program provided data that formed the foundation for the GMR 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 years 2007 through 2010. The project used a genetic mark-recapture (GMR) protocol developed by Rawding et al. (2012) 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 that 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 GMR described by Rawding et al. (2012), 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. Adult and juvenile genetic sampling prior to 2011 was opportunistic and directed towards coded-wire-tag (CWT) recovery, and tribal monitoring and research goals in the Stillaguamish River unrelated to SSP.

Spawner tissues (fin clip or scale) are 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 smolts, and fin clips are taken from a subset of wild smolts.

Collections and genotyping

We genotyped Chinook salmon spawner carcass samples collected in 2011 (see Table 1 for all collections) and genotyped smolts sampled from a mainstem smolt trap in 2011. To increase the number of spawner genotypes for each brood year we genotyped scale samples collected from natural spawners in 2007- 2010 and added those genotypes to existing data collected previously for Tribal monitoring purposes. Other existing data from the monitoring program that was used in this study included genotypes for smolts collected in the mainstem trap in years 2008, 2009, and 2010. We also genotyped tissue samples collected from hatchery

broodstocks 2007, 2008, and 2010 (2009 broodstock had been genotyped previously) to help identify unmarked hatchery juveniles (juveniles that left the hatchery without receiving an adipose fin clip). Smolts collected in the mainstem in years 2008 – 2011 were assigned back to natural spawners and hatchery broodstocks from 2007-2010 (juveniles were assigned to their brood year parents) for the GMR 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 (using FSTAT), heterozygosity, genetic diversity 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 collection 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 GMR, 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 GMR 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 with an efficient algorithm within minutes, 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.

We used COLONY as another 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 are unavailable from FRANz. COLONY has three options for run length (short, medium and long), and we chose medium runs for all brood years to strike a balance between obtaining results in a timely manner and consistency in the results. (Short and medium runs differed at least five-fold in duration, e.g. 2 days versus 12 days, respectively, and long runs never converged) We compared results from FRANz and COLONY and used the FRANz results for binomial Lincoln-Peterson calculations following Seber (1982):

$$N = \frac{M(C+1)}{(R+1)}$$

Where:

N = adult escapement

M = marks – adult carcasses from spawning areas that were genotyped successfully

C = captures - wild-born smolts that were captured at the smolt trap and genotyped successfully

R = recaptures - carcass assigned to a juvenile (1 recapture if 1 parent assigned, 2 recaptures if 2 parents assigned).

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):

$$\text{Var} = \frac{M^2(C+1)(C-R)}{(R+1)^2\,(R+2)}$$

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):

$$N = \frac{(M+1)*(C+1)}{(R+1)} - 1.$$

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

$$\text{Var} = \frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2(M+2)}$$

For a third method to estimate spawners, we used the COLONY results in accumulation curves that described the unique number of breeders giving rise to the juvenile sample, following Petit and Valiere (2006). While the binomial and hypergeometric provide a spawner abundance estimate, the accumulation curve estimates the number of successful breeders. Todd Seamons (WDFW) wrote an R script 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 used the Beverton-Holt (BH) spawner-recruit model (Beverton and Holt 1956) and Continuous Smooth Hockey Stick (CSHS) model (Froese 2008) to generate accumulation curves that reached asymptotes at the maximum estimates of unique spawners and generated confidence intervals for these estimates.

Potential sources of bias

Unmarked hatchery juveniles were one potential source of bias. Up to 5% of juveniles leave the hatchery with their adipose fin intact (un-clipped) (Charlotte Scofield, Stillaguamish Tribal 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.

A key assumption for an unbiased Petersen estimate is that all fish in the population have the same probability of being tagged, or that all fish have the same probability of being captured in the second sample, or that marked fish mix uniformly with unmarked fish. Rawding et al (2012) developed a GMR study design that sampled outmigrants proportional to their abundance to meet the assumption that all fish have the same probability of being captured in the second sample. In the Stillaguamish River, smolt sampling was interrupted by weather events and debris in the smolt trap, preventing sampling proportional to juvenile outmigration in some weeks (Figure 2-5), thus precluding the application of this study design. However, the pooled Petersen estimate remains valid if marked fish mix uniformly with unmarked fish. This assumption can be tested with a χ^2 test. This tests the hypothesis that there is no difference in proportions of recaptured genotypes among sample periods, often referred to as the "equal proportions" test (Schwarz and Taylor 1998). However, comparisons of the χ^2 statistic to the χ^2 distribution can produce misleading results when sample sizes are small. Therefore, weekly genotypic data were pooled into equal periods to ensure that expected frequencies were five or greater per time strata (Quinn and Keough 2002).

To explore further how opportunistic rather than proportional juvenile sampling affected GMR abundance estimates, we developed subsampling protocols for each outmigration year based on weekly outmigrating juvenile abundances. The protocol looked at the proportion of juveniles outmigrating each week and calculated the number of juveniles that should be subsampled each week to achieve proportional sampling for each week (see Figure 2 - 5). Todd Seamons (WDFW) wrote an R script that bootstrapped the subsampling each week 10000 times and calculated *N* with the Lincoln-Peterson equation to generate distributions and confidence intervals for mean *N* values.

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.

Results

Collections and genotyping

Genotype abundance for juveniles and carcasses varied among brood years (Table 3). Because most of the sampling was conducted prior to developing research objectives for GMR, only natural-origin carcasses were sampled in spawning areas. Juvenile sampling occasionally matched actual outmigration proportions in some weeks each year (Figure 2 - 5). We had mixed success in genotyping spawner scale samples; success per collection ranged from 8% to 40%, averaging around 27% (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 GMR. Genotyping success for hatchery broodstock tissue samples averaged 72% (following the same protocol of reruns and re-

extractions when samples failed). Success was higher for broodstock samples because they were collected from living fish rather than decayed carcasses. Genotyping success was highest for juvenile samples, but there were still some juveniles each year without genotypic data. Less than 10% of juvenile samples (eg. 24/597 in 2011) 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 spawner collections were random samples (Table 4); there were no significant deviations from Hardy-Weinberg equilibrium (HWE). Genetic statistics also indicated that juvenile collections included families: linkage disequilibrium was high even after correcting for multiple tests.

Identifying unmarked hatchery juveniles and yearlings

We identified unmarked hatchery juveniles in each collection year (Table 1) and confirmed that putative hatchery juveniles had no parents among the natural spawners by conducting assignment tests without hatchery broodstocks in the parent pools. Because roughly 25% of the hatchery broodstock were below our 10 locus data threshold, our method potentially missed some unmarked hatchery juveniles. However, GMR results suggested that unmarked hatchery juveniles had minimal impact on *N* calculations (presented below).

Regression plots showed that there were a few putative yearlings mixed in with sub-yearlings (Figure 8). In years where we had collection identification codes associated with length data (2009 – 2011), we removed suspected yearlings. In 2008 data we decreased the capture number by six to adjust for suspected yearlings.

Genetic Mark Recapture

The number of assignments using FRANz and COLONY were congruent in most brood years. 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 tended to assign a few more parents than COLONY, likely because COLONY restricted some assignments with genotypes created for unsampled parents.

Spawner estimates

The *N* estimated from the bootstrap subsampling protocol (proportional juvenile subsampling) tended to be higher and have greater variance than estimates based on pooled juvenile samples (Table 5, Figure 6). This was because there was less data per week after corrections for proportional sampling. However, the Equal Proportions χ^2 tests indicated no difference in assignments among time strata in any year (see Appendix 1 for assignments over collection period), so we could use pooled juvenile data without subsampling to obtain spawner abundance estimates based on binomial sampling ($p > 0.05$ for difference in assignments among time strata each sampling year). In estimates with pooled data *N* values were slightly higher when unmarked hatchery juveniles were included (hatchery juveniles increased capture numbers), but there were few hatchery juveniles so differences were minimal and confidence intervals encompassed *N* calculated without hatchery juveniles. Because we genotyped fewer than 100% of the hatchery broodstocks, some unmarked hatchery juveniles remained unidentified. However, they appeared to impart a minimal bias to abundance calculations – *N*

values calculated without removing suspected unmarked hatchery juveniles ranged from 1% to 6% higher than values calculated after removing suspected unmarked hatchery juveniles.

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 each 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. With the exception of the 2009 brood year, the hypergeometric estimates were lower than binomial estimates (Table 5, Figure 6). For the 2009 brood year, although within the confidence intervals of the binomial estimates, the hypergeometric estimate was higher than other estimates due to fewer recaptures by COLONY than FRANz for the 2009 brood year.

Accumulation curve

Accumulation 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 accumulation curve estimates to be less than GMR estimates. For the 2007 brood year (Figure 7), the point estimates from the accumulation curves were far below the binomial estimates (Table 5, Figure 6). For the 2008 brood year, the point estimates from the accumulation curves were roughly half the estimates of the binomial. For the 2009 brood year, the bounds for the Beverton-Holt (BH) accumulation curve estimate encompassed the estimates from the binomial (Table 5, Figure 6, Figure 7). For the 2010 brood year, the point estimates from the accumulation curves were roughly 60% of the redd count estimate and 40% of the binomial estimate (Table 5, Figure 6, Figure 7). In all years the BH estimates were higher than the Continuous Smooth Hockey Stick (CSHS) estimates.

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 can 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). Violation of these assumptions bias calculated 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 juveniles assigning to spawner parents as

"recaptures" of the parent genotype in the second sampling. But for calculated *N* to be valid, we must meet the basic assumptions. Because conditions and sampling varied among brood years and we used multiple techniques for calculating *N*, we could hypothesize how data may have violated some assumptions and assess how those violations affected calculated *N*.

Our data meet some assumptions completely (system is closed if hatchery fish are detected) and violate other assumption with the violations vary 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. 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* calculation 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 their genotype indicates better quality DNA. However, this 10 locus threshold introduced another upward bias 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. Our spawner samples were skewed towards females in 2007 and 2009, towards males in 2008, and roughly equal in 2010, suggesting that there could be differences in carcass recovery based on sex. Further, if weather or other factors prevent regular spawner surveys, spawners during different portions of the run could be flushed from the system and thus uncatchable. 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. Weather events also interfered with juvenile sampling as the smolt trap is inoperable in high water. Missing trap days would affect our estimates if the proportion of marked fish was different between periods when the trap was and was not operated. However, our study addressed equal catchability by testing the hypothesis that there is no difference in the marked proportion of outmigrants (recaptures or assignments to parents) in the second sampling period, which minimizes concerns about sex and size bias in carcass recoveries (Zhou 2002 and Murdoch et al. 2009), and differences in individual reproductive success.

Another possible source of bias could arise from sampling only unmarked, presumably natural-origin spawners for brood years 2007 to 2010 (starting 2011 samples are collected from all spawners). However, Rawding et al. (2012) 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. Therefore, our natural-origin only carcass collections should not bias estimates of *N*.

We have addressed objective 1, estimate spawner abundance, the main focus of the original proposal. However, we were unable to address objective 2 (estimate natural spawner abundance by origin, natural or hatchery) because carcass samples were obtained prior to this study. We have described differences between mark-recapture and redd-based abundance estimates. As noted above, some of the difference among estimates arises from poor environmental conditions when redd observations are less than 100% and redd counts may be a minimum estimate. We are exploring methods to account for redds missed in surveys and to account for poor survey conditions. These will be presented in subsequent reports.

Comparison among techniques

As noted above, the binomial and hypergeometric estimates were similar and differences were greatest when there were few recaptures. However, we recommend using the binomial estimator because it uses all available mark-recapture data. 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 estimates with estimates from accumulation curves and expanded redd counts (Figure 6, Table 5). The comparisons varied in different ways each year. In 2008 and 2009 the redd-count estimate was within the CI from the binomial estimate, and in 2007 and 2010 it was not within the CI from the binomial estimate. The binomial CI overlapped the accumulation curve CI in only 2009. This lack of concordance 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 weather. For example, in 2007 high water challenged redd counting during all of October which probably led to an underestimate in the redd count. Also, continued high flows flushed carcasses from the system making carcass recovery difficult, resulting in few genetic "marks" for that year (Table 3). 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 GMR methods were similar to each other and the smaller confidence intervals encompassed the redd count estimate. In the 2009 brood year, there was concordance between GMR estimates and redd count estimates, confidence intervals were small, and the CV was 5%. In the 2010 brood year, lower proportions of spawners and juveniles were sampled, GMR estimates varied, confidence intervals were larger, and the CV was 15.1%, which was high but within the SSP goals. Among all years, sampling was best for the 2009 brood year with a high number of marks, captures and recaptures. The GMR estimate (~1100 spawners) suggested that at least 10% of the estimated number of spawners had been sampled (147 marks).

As noted above, the BH estimates were always higher than the CSHS estimates of successful breeders. This is because the BH estimate is based on unlimited number of juveniles. In reality, carrying capacity of the Stillaguamish system limits the number of juveniles. Because the BH model would likely overestimate the number of successful breeders (Petit and Valiere 2006), we recommend using the CSHS model unless very few juvenile are collected.

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 definitively identify unmarked hatchery juveniles. Using this method, some unmarked hatchery juveniles were likely undetected because we lacked genotypes for their parents. However, results suggested that unmarked hatchery juveniles had little impact on GMR 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 calculated *N*.

Conclusions

Our data suggests that GMR is a useful tool for estimating Chinook salmon escapement in the Stillaguamish River. Based on binomial sampling, the GMR estimates met CTC standards for precision in three of four years. Given that juvenile sampling efforts are standardized, we recommend increasing adult tissue sample collections to consistently meet precision standards. The CTC standards for unbiased estimates were largely achieved. Some concern remains regarding the assumption that marks are correctly identified and reported, which is a genetic assignment concern. WDFW is pursing simulations and double sampling to address this. In addition, increasing adult sample sizes should improve genetic assignments. This GMR project was very successful given the opportunistic design of the program.

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Table 1. List of samples in analyses with number of samples analyzed (N) and the number of samples with at least 10 loci in their genotype (N>9 loci).

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	52		
09CS	North Fork Stillaguamish summer (spawning grounds)	adult	183	138		
09NB#	North Fork Stillaguamish summer (spawning grounds)	adult	139	4		
10DD	North Fork Stillaguamish summer (spawning grounds)	adult	70	52		
10NW#	North Fork Stillaguamish summer (spawning grounds)	adult	12	0		
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		
07NH#	South Fork Stillaguamish fall spawners	adult	13	4		
08LD	South Fork Stillaguamish fall spawners	adult	13	6		
08HX#	South Fork Stillaguamish fall spawners	adult	10	5		
09CT	South Fork Stillaguamish fall spawners	adult	5	5		
10CZ	South Fork Stillaguamish fall spawners	adult	2	2		
08LE	Mainstem Stillaguamish	smolt	567	508	14	6
09CQ	Mainstem Stillaguamish	smolt	799	757	8	5
10DA	Mainstem Stillaguamish	smolt	1315	1232	33	5
11BU#	Mainstem Stillaguamish	smolt	597	544	29	0

samples added under Sentinel Stocks project in 2011, other samples were genotyped prior to this study for Tribal monitoring and research

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
	Ssa197a		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 2007-2010 using genetic mark-recapture (GMR). 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 GMR estimate is under "CV". Redd-based abundance estimates were from expanded redd counts. GMR 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 count 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	1308	15%	783

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), 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. South Fork Stillaguamish River spawner collections were too small for meaningful calculation of genetic statistics.

				heterozygosity deficit	heterozygosity excess	linkage (91 pairs)	
	Gene Div	Rich	F_{IS}	*p* value	*p* value	5%	1%
07NF spawners	0.8741	14.79	-0.005	0.597	0.404	5	0
08NF spawners	0.8672	14.32	0.002	0.440	0.561	3	1
09NF spawners	0.8731	13.99	-0.009	0.844	0.156	24	8
10NF spawners	0.8654	14.29	0.002	0.443	0.557	3	1
07NF broodstock	0.8727	14.11	-0.007	0.737	0.264	7	1
08NF broodstock	0.8661	14.18	0.005	0.322	0.679	10	3
09NF broodstock	0.8696	13.98	-0.012	0.909	0.091	15	2
10NF broodstock	0.8703	13.88	0.005	0.258	0.743	12	4
08 Mainstem smolts	0.8736	14.51	-0.002	0.652	0.351	85	70
09 Mainstem smolts	0.8764	14.44	0.002	0.303	0.699	89	77
10 Mainstem smolts	0.8717	14.02	-0.001	0.565	0.437	91	83
11 Mainstem smolts	0.8764	14.41	-0.008	0.982	<u>0.018</u>	88	69

Table 5. Summary of abundance estimates for all brood years using different methods (see Figure 6).

		pooled, all juv in	pooled, no H juv	subsample, no H juv	hypergeometric	accumulation curve	redd
2007	*N*	2157	1954	2560	1291	435	616
	Var	269340	208427	846478	137994		
	CV	24.06%	23.36%	35.94%	28.77%		
2008	*N*	1935	1914	2098	1711	676	1671
	Var	63244	61881	497354	96936		
	CV	12.99%	12.99%	33.61%	18.20%		
2009	*N*	1100	1061	901	1239	979	1001
	Var	3158	2921	7135	6888		
	CV	5.11%	5.09%	9.38%	6.70%		
2010	*N*	1508	1308	1266	837	488	783
	Var	54804	39092	113777	17076		
	CV	15.53%	15.12%	26.64%	15.62%		

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

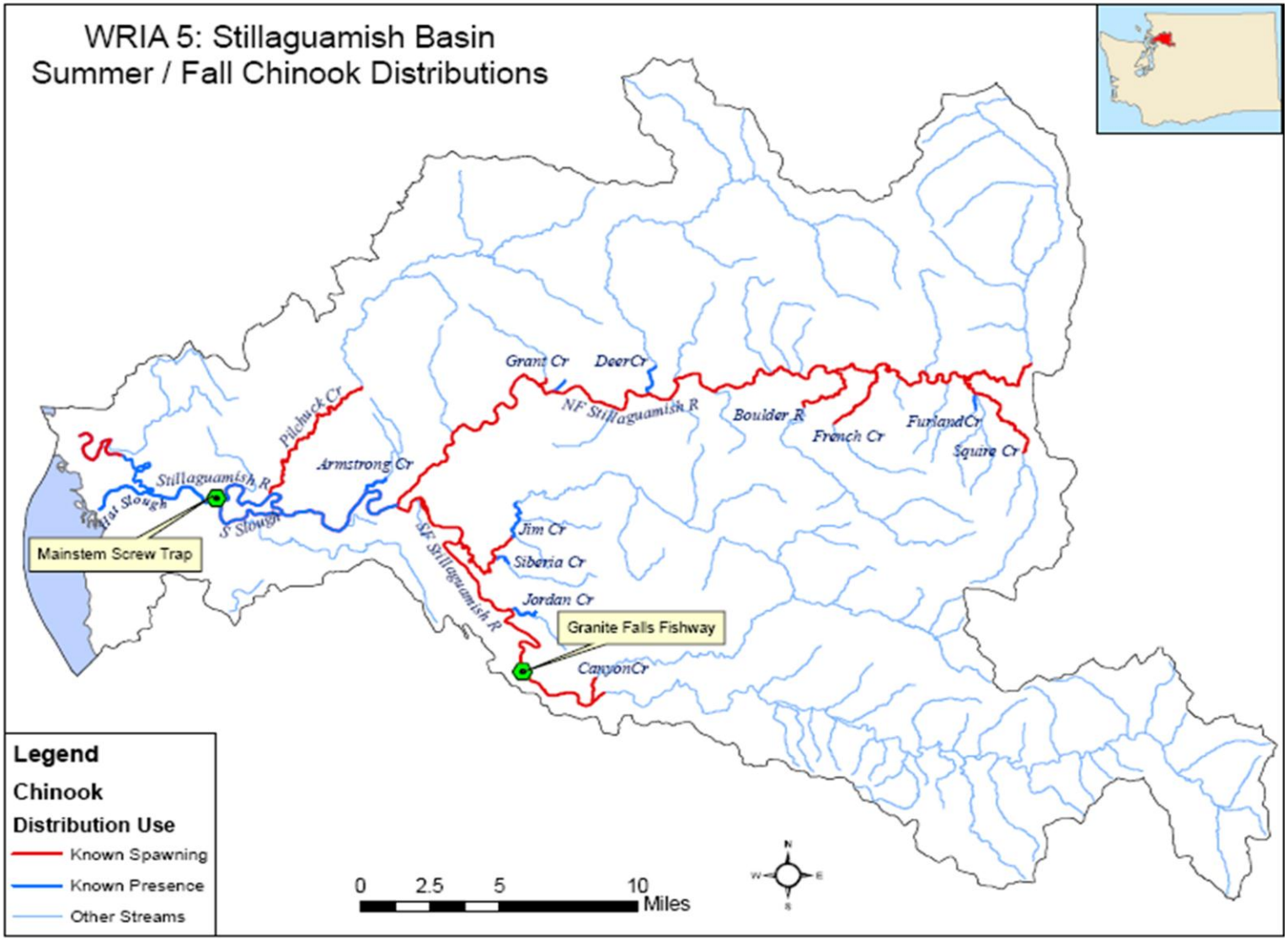


Figure 2. Subsampling to correct proportions of weekly out-migrants in 2008. Red bars in top and bottom plot are the actual number of juvenile samples per week. Blue bars in top plot are the estimated number of out-migrants per week and the green bars in the bottom plot are the estimated number of subsamples to match out-migration proportions.

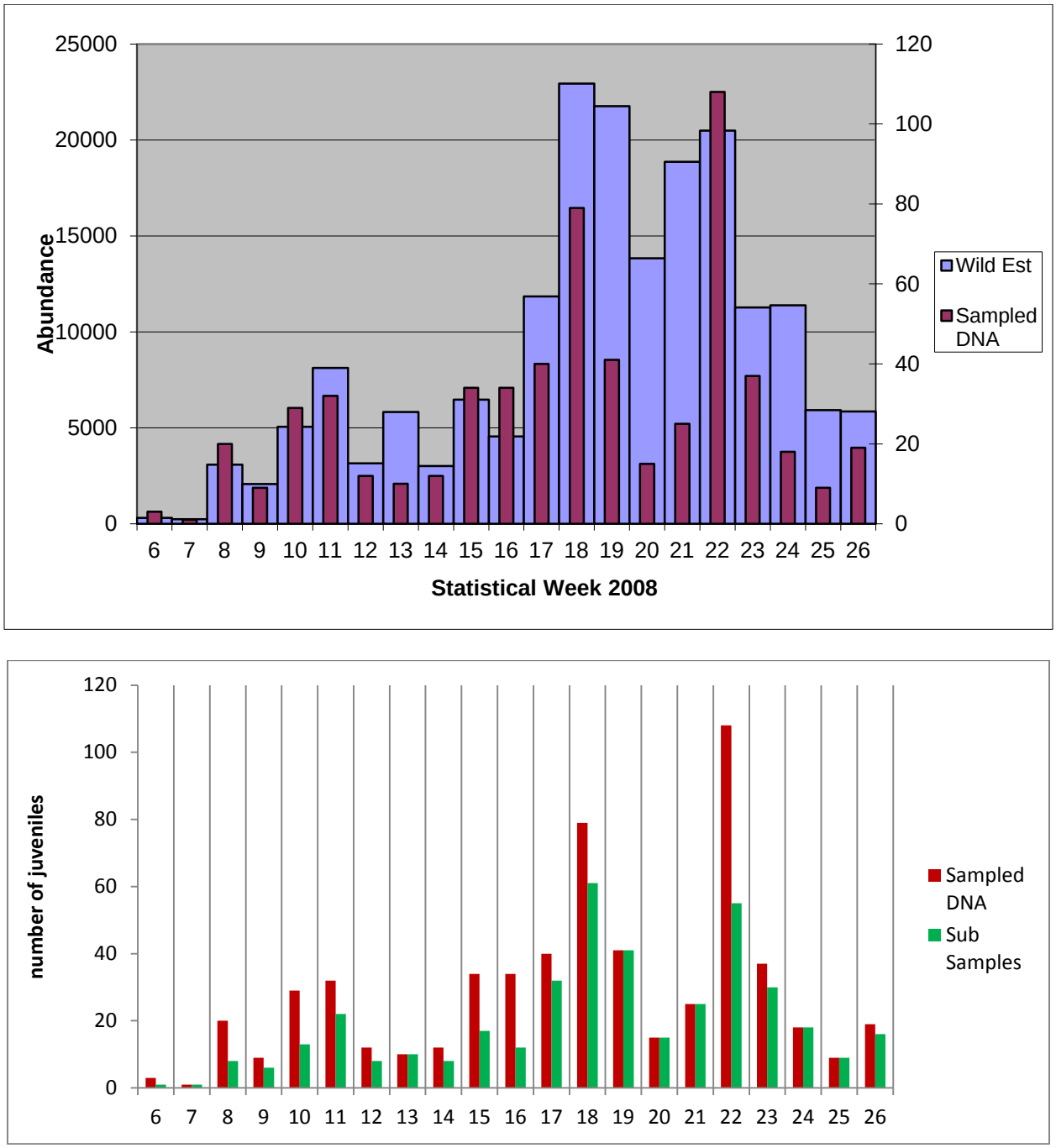


Figure 3. Subsampling to correct proportions of weekly out-migrants in 2009. Red bars in top and bottom plot are the actual number of juvenile samples per week. Blue bars in top plot are the estimated number of out-migrants per week and the green bars in the bottom plot are the estimated number of subsamples to match out-migration proportions.

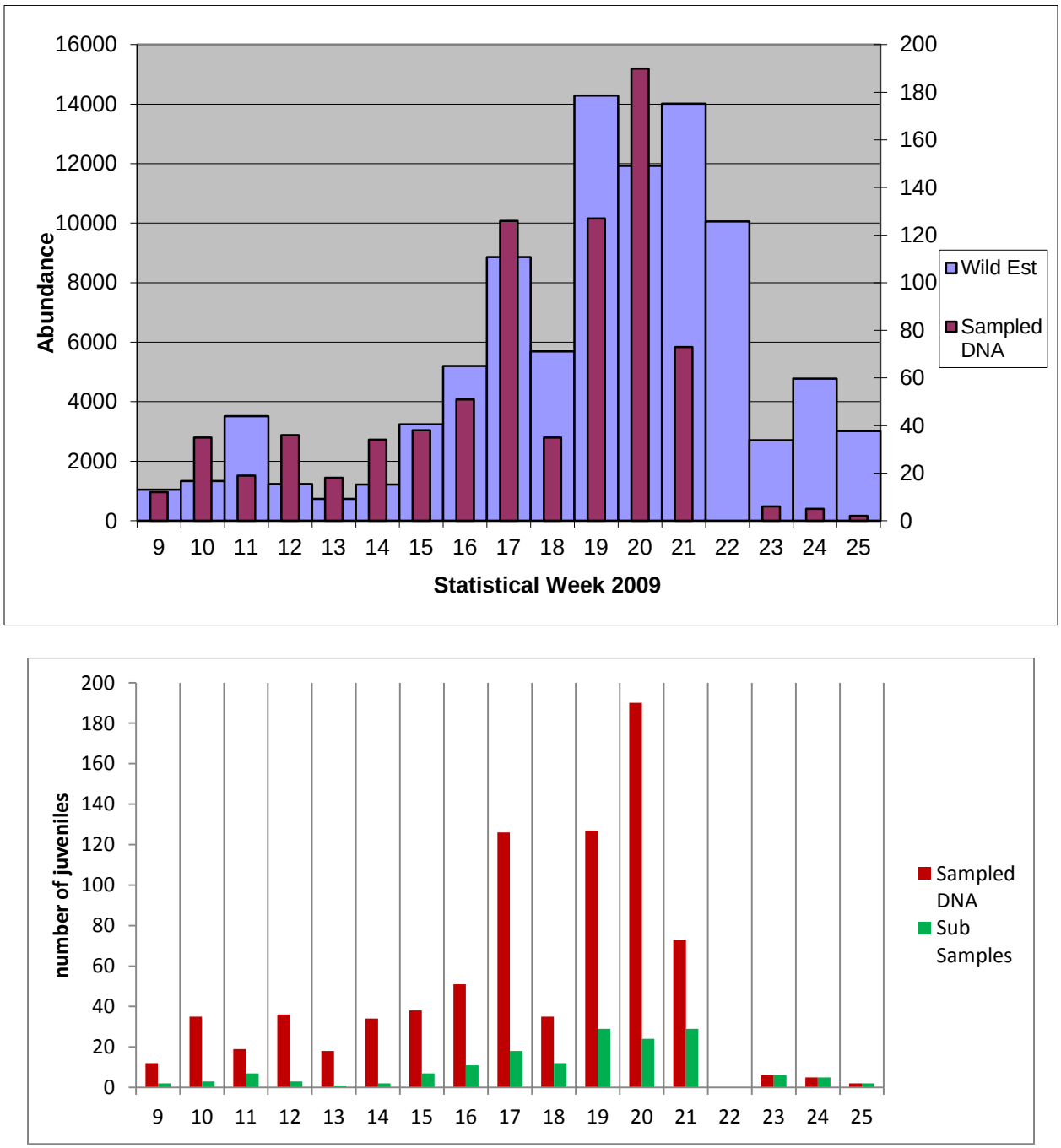


Figure 4. Subsampling to correct proportions of weekly out-migrants in 2010. Red bars in top and bottom plot are the actual number of juvenile samples per week. Blue bars in top plot are the estimated number of out-migrants per week and the green bars in the bottom plot are the estimated number of subsamples to match out-migration proportions.

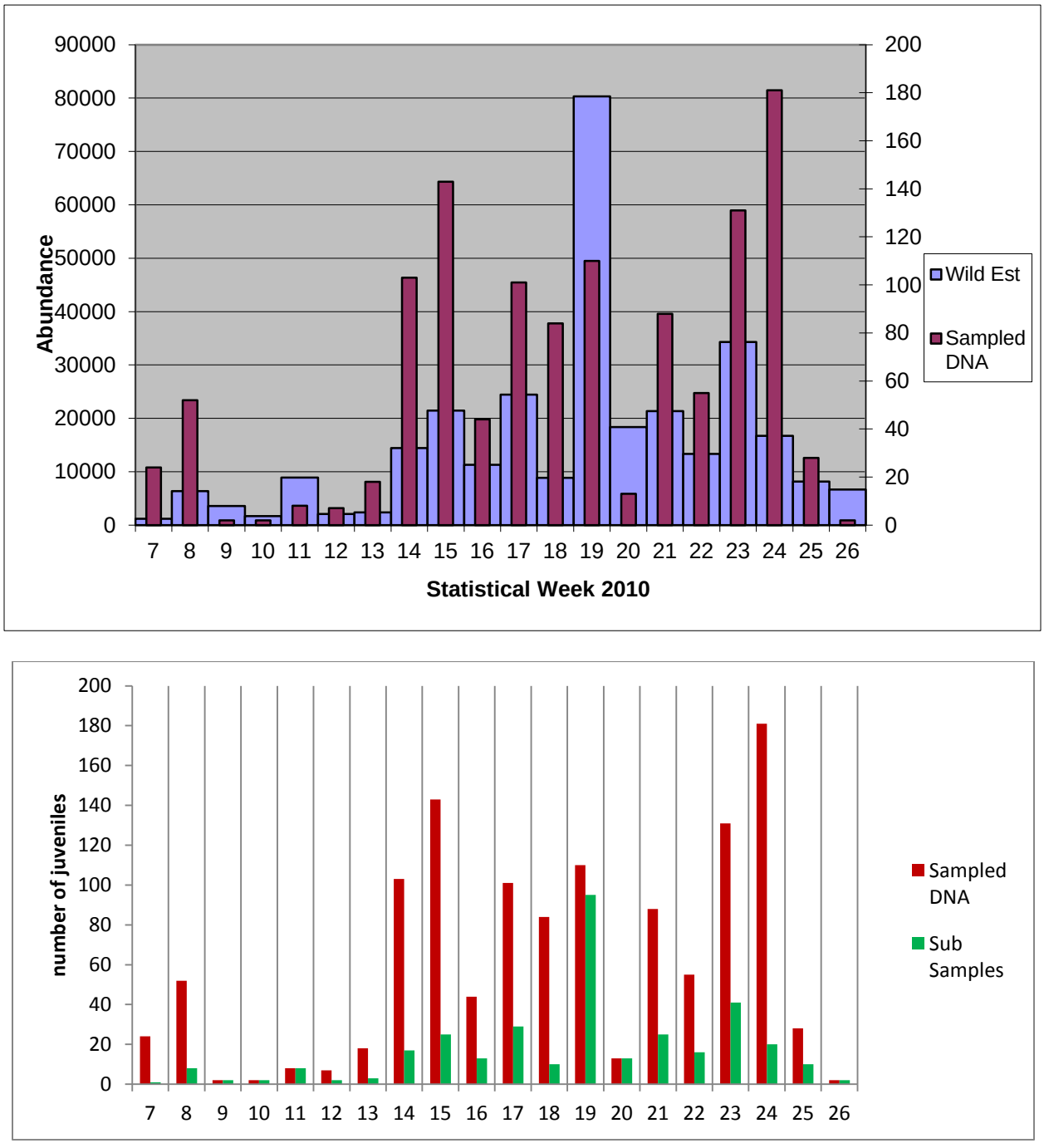
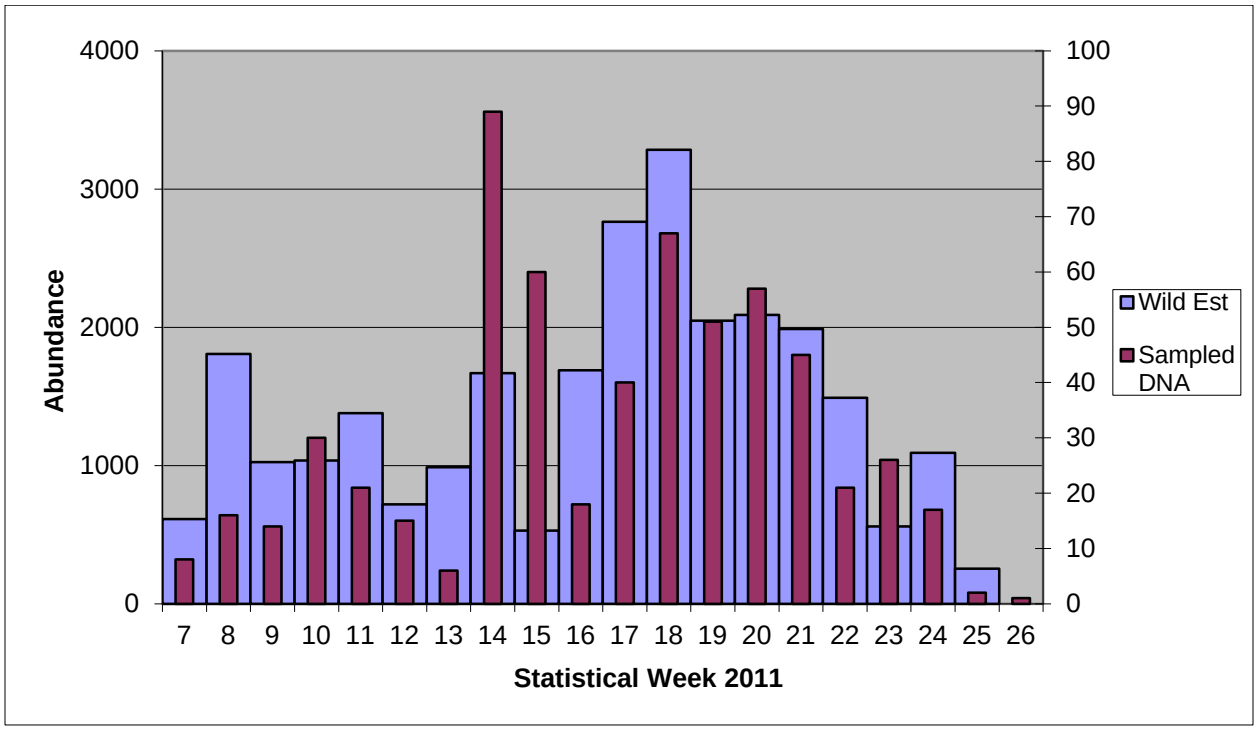


Figure 5. Subsampling to correct proportions of weekly out-migrants for 2011. Red bars in top and bottom plot are the actual number of juvenile samples per week. Blue bars in top plot are the estimated number of out-migrants per week and the green bars in the bottom plot are the estimated number of subsamples to match out-migration proportions.



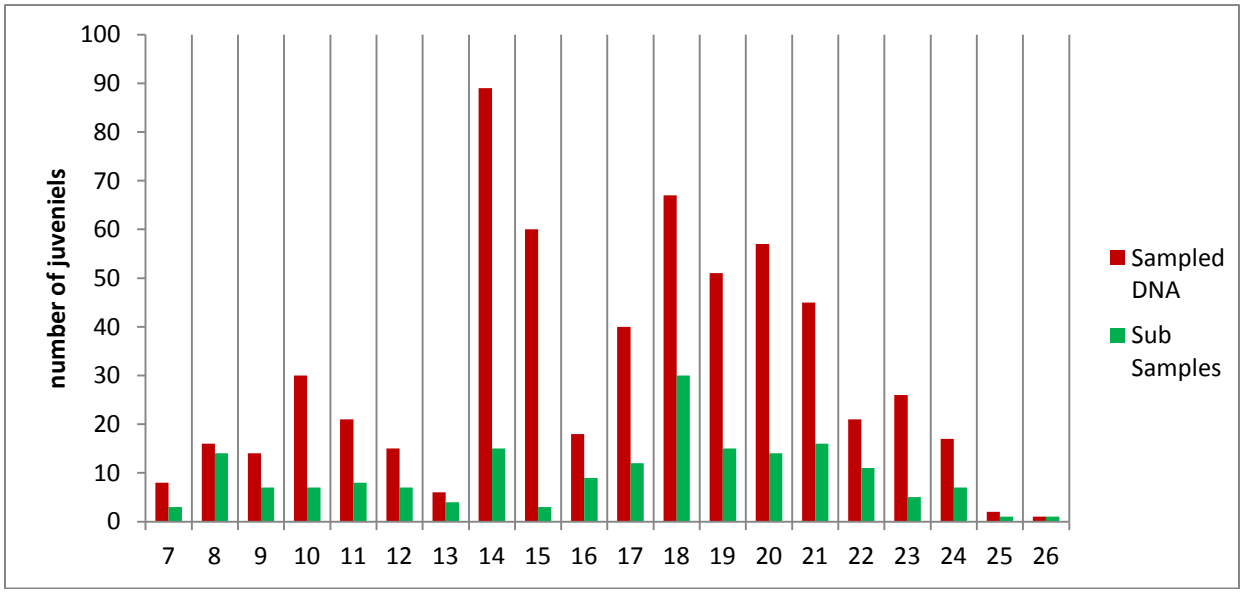
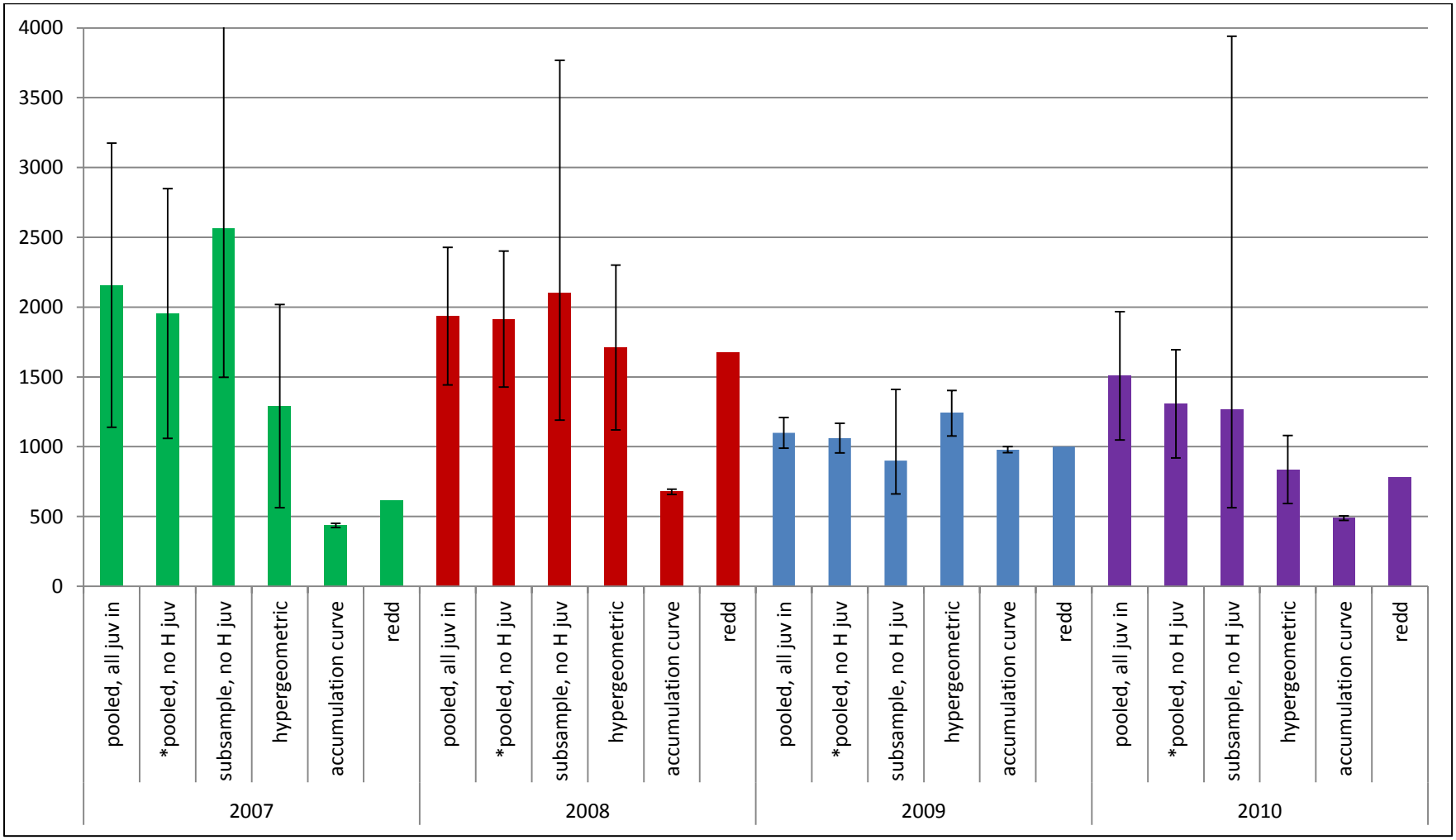


Figure 6. Comparison of the abundance estimates with different methods: pooling all juveniles without removing hatchery and yearling juveniles (pooled, all juv in), pooling all juveniles and removing hatchery and yearling juveniles (pooled, no H juv), subsampling juveniles by week after removing hatchery and yearling juveniles (subsample, no H juv), hypergeometric with hatchery and yearling juveniles removed (hypergeometric), accumulation curve (with hatchery and yearling juveniles removed) and redd count expansion (redd). Actual confidence interval for the 2007 "subsample no H juv" extended to 4493.



* pooled binomial estimates are presented in Table 3.

Figure 7. Accumulation curves estimating spawners in brood years 2007, 2008, 2009 and 2010. The large dots are the averages over 10,000 re-samples.

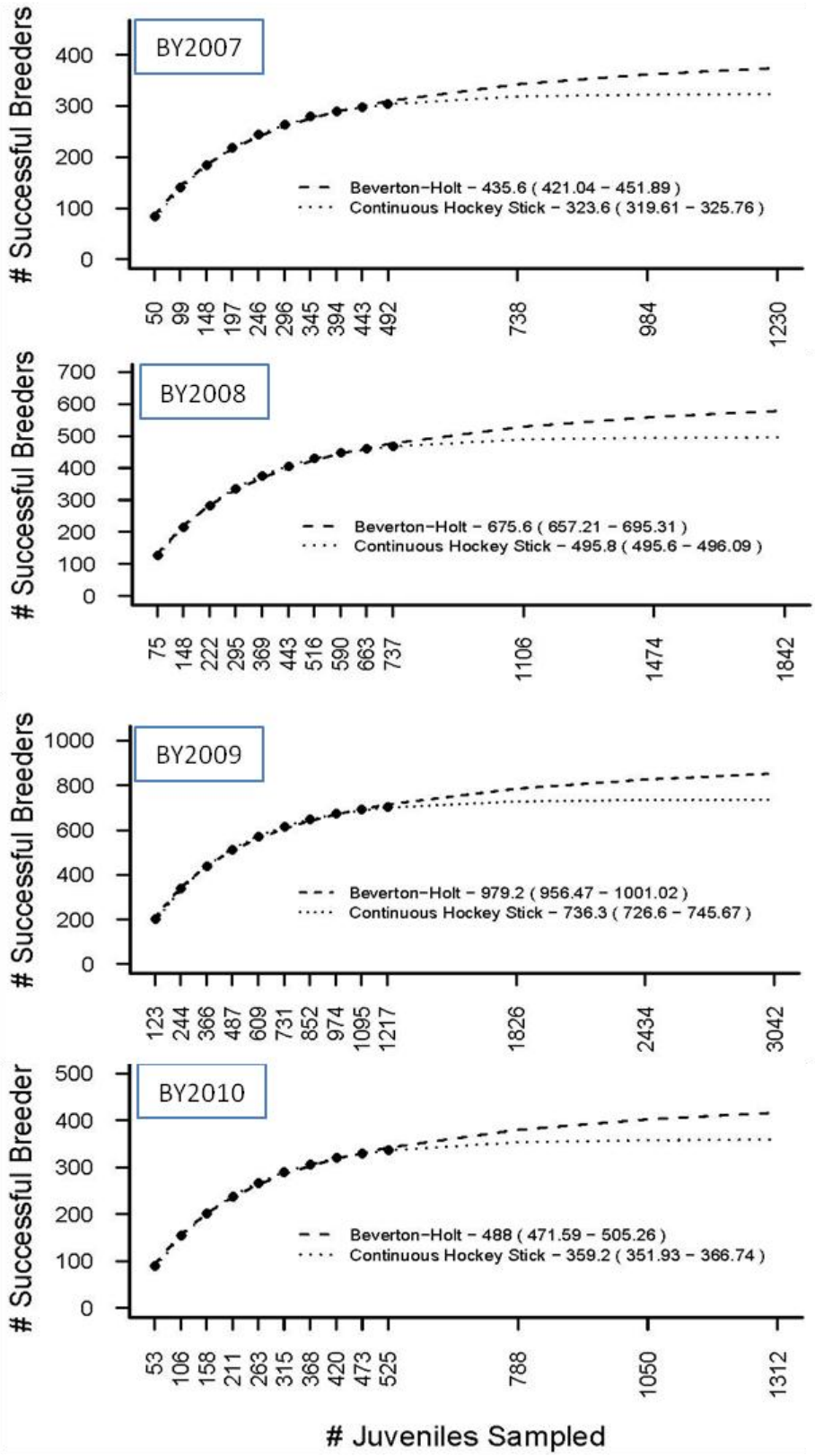
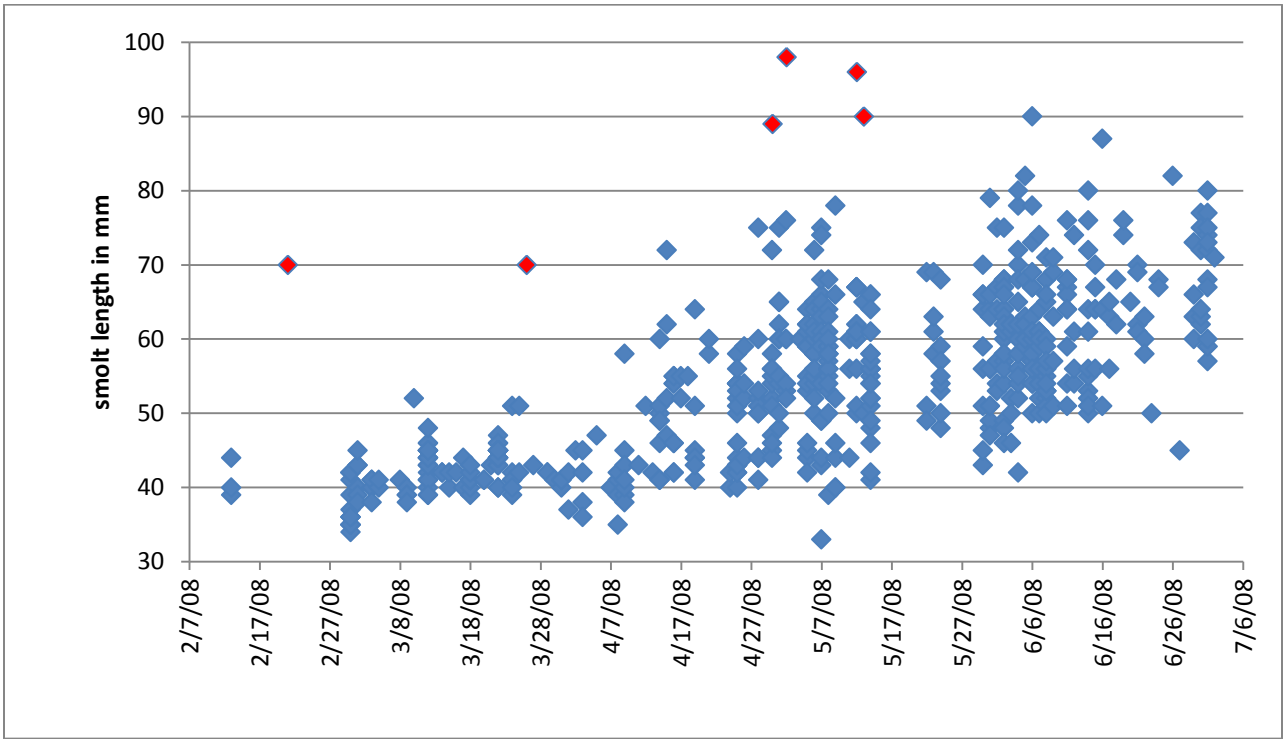


Figure 8. Regressions of smolt length on out-migration dates. Suspected yearlings are in red. Note: there was no DNA sample for suspected yearling collected February 24, 2009.



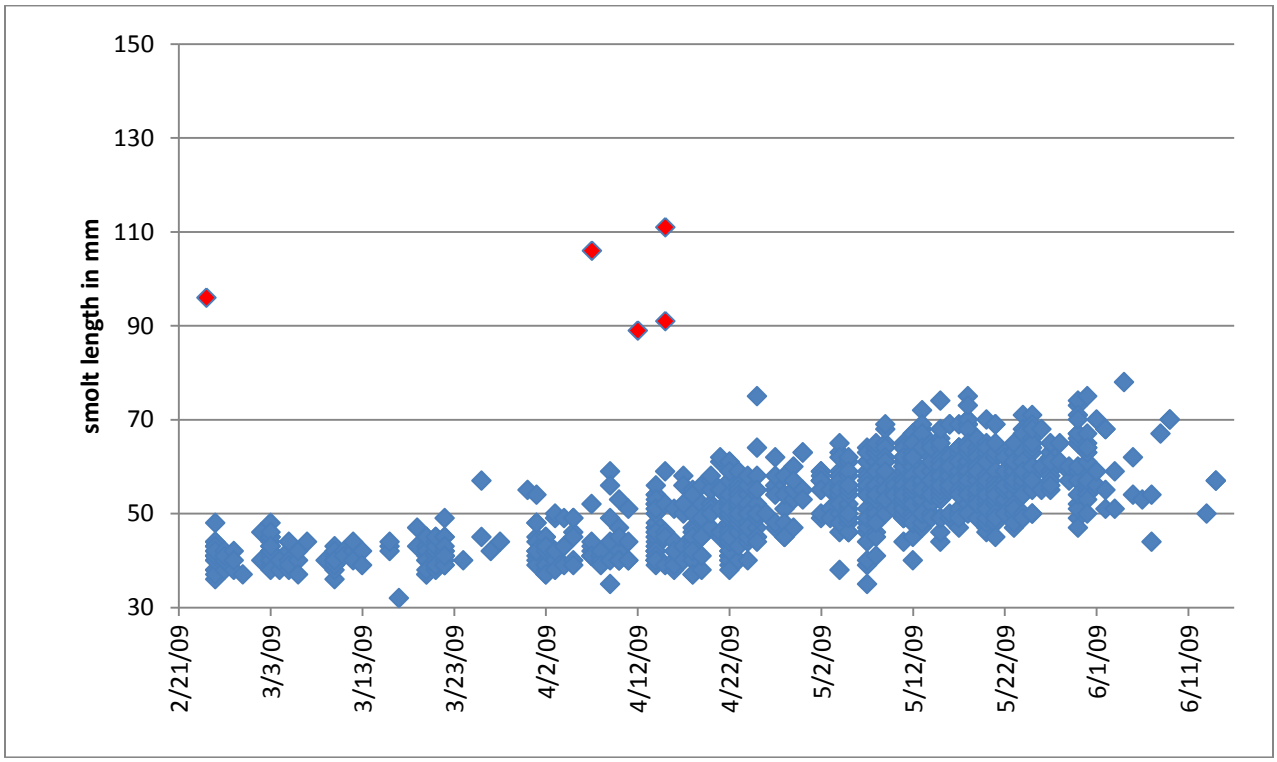
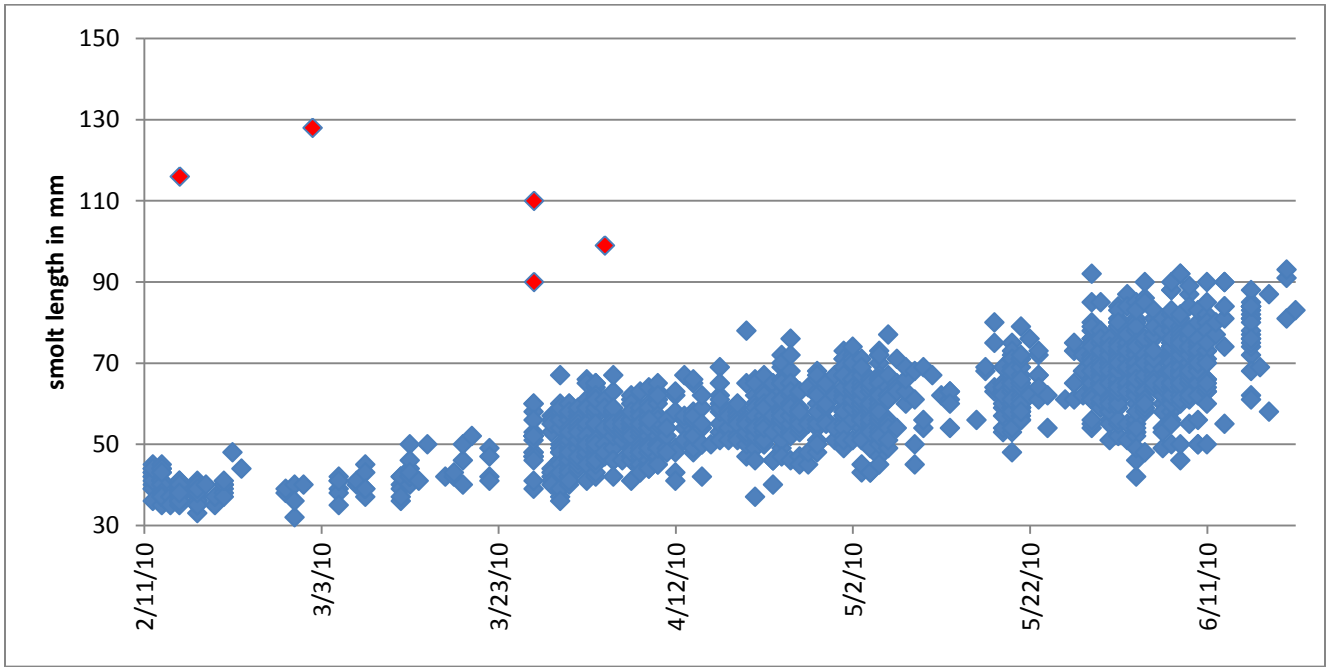
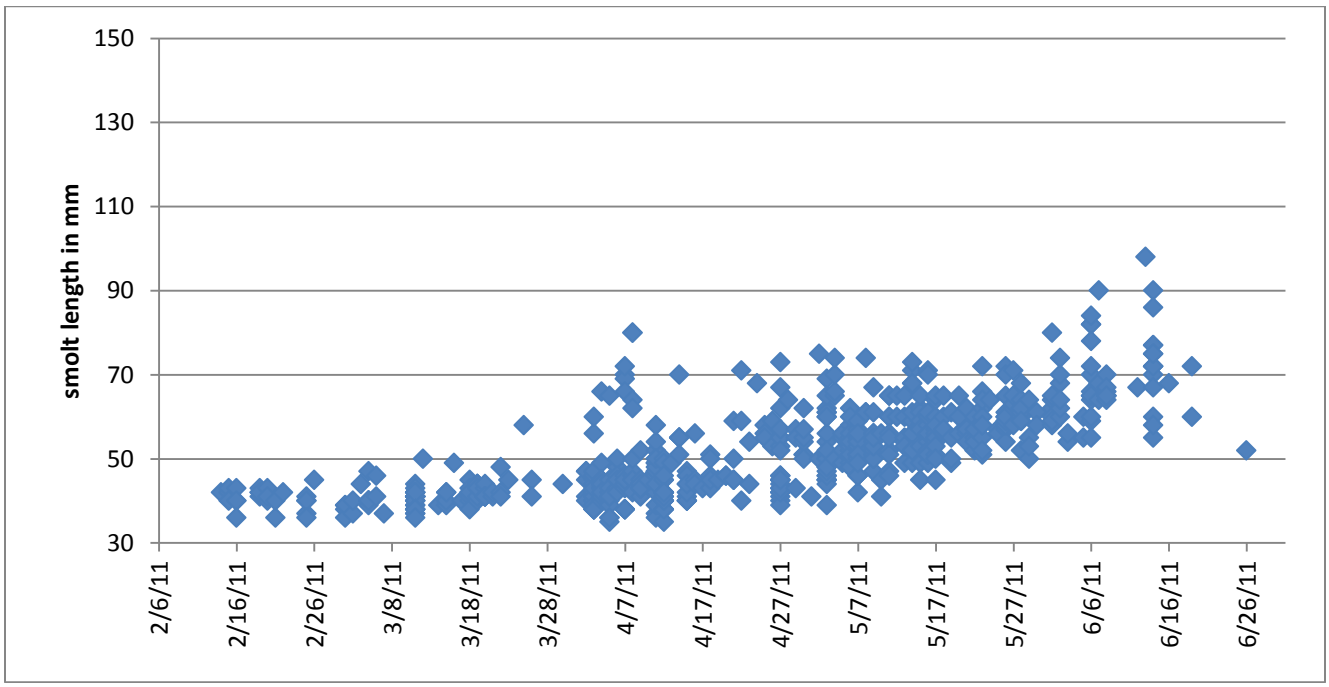


Figure 9 continued. Regressions of smolt length on out-migration dates. Suspected yearlings are in red.





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

Brood year 2007

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	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