Confidence Intervals f or Run Composition

of Returning Salmonids

##### Kirk Steinhorst

Department of Statistical Science

University of Idaho, Moscow, ID 83844-1104

email: [kirk@uidaho.edu](mailto:kirk@uidaho.edu)

Tim Copeland2

Michael W. Ackerman3

Eric C. Anderson4

William C. Schrader2

##### May 5, 2014

2Idaho Department of Fish and Game, Nampa Fisheries Research, 1414 East Locust Lane, Nampa, Idaho, 83686, USA

3Pacific States Marine Fisheries Commission & Idaho Department of Fish and Game, Eagle Fish Genetics Lab, 1800 Trout Road, Eagle, Idaho, 83616, USA

4Fisheries Ecology Division, Southwest Fisheries Science Center, 110 Shaffer Road, Santa Cruz, California, 95060, USA

##### ABSTRACT

We propose weighted estimators of fish composition for wild steelhead and Chinook salmon returning to a single point in a river system. Using data on overall fish numbers derived from counts at an observation window, trap data providing separation of wild and hatchery fish, and data on sex, age, and genetic stock of wild fish, we propose one-at-a-time and simultaneous confidence intervals for sex or age or genetic stock based on parametric bootstraps of both the wild/hatchery data and the sex/age/stock data. Using two simulations, we show that the estimators are generally unbiased with confidence intervals that have good coverage. We also show that if the numbers of fish returning for a given category are not too small, then we can estimate the true number returning within 10% with 90% confidence.

KEYWORDS: Bootstrap, Stratified Sampling, Salmonid Escapement, Simultaneous Inference, Genetic Assignment Test

1. INTRODUCTION

Migratory life histories are important in fisheries management and conservation (Hilborn and Walters 1992). Given migratory characteristics, the spatial distribution of a stock or population is predictable, which facilitates exploitation and also sampling for research and management. Sampling may be conducted by test fisheries (e.g., Flynn and Hilborn 2004, Beacham et al. 2012), hydroacoustics (e.g., Tarbox and Thorne 1996, Pritt et al. 2013), or by other means. A highly controlled sampling regime can be instituted by counting or sampling migrating fish as they move past barriers such as weirs or dams (e.g., Wagner 2007). In all of these sampling scenarios it is typical to subdivide overall abundance into groups of management interest by applying compositional data (e.g., species, stock, age, and sex) derived either from the primary sampling gear or by a secondary sampling gear (e.g., using gill net samples to allocate hydroacoustic counts to species, Rudstam et al. 2012). The complexities of fisheries sampling programs and the relevant groups into which the fish are parsed present difficulties for estimating precision about the point estimates generated.

Here we present a case study with management and conservation applications. Populations of steelhead trout *Onchorhynchus mykiss* and Chinook salmon *O. tshawytscha* in the Snake River basin of Idaho, NE Oregon, and SE Washington declined substantially following the construction of hydroelectric dams in the Snake and Columbia rivers. Raymond (1988) documented a decrease in survival of emigrating steelhead trout and Chinook salmon from the Snake River following the construction of dams on the lower Snake River during the late 1960s and early 1970s. Abundance rebounded slightly in the early 1980s, but then escapements over Lower Granite Dam into the Snake River basin declined again (Busby et al. 1996). As a result, Snake River spring-summer Chinook salmon were classified as threatened in 1992 under the Endangered Species Act (ESA); steelhead were listed as threatened under the ESA in 1997. In recent years, abundances in the Snake River basin have slightly increased. However, the increase has been dominated by hatchery produced fish (intended to mitigate for lost fisheries and to supplement natural populations), while the returns of steelhead and Chinook salmon born in the natural environment remain low.

Fisheries biologists need to know how many wild versus hatchery salmon and steelhead return in order to properly manage fisheries as well as to assess the conservation status of wild populations. In our case, we are particularly interested in the number of wild fish returning. Further, we want to know numbers returning by sex, age, and genetic stock. There are two approaches to determining run composition (Starr and Hilborn 1988): 1) Direct identification of fish from tags, scales, genetic analysis, and/or morphology, and 2) Reconstruction of the run from catch data, adult escapement numbers (numbers of fish returning from the ocean to a given point), and migration paths (Prvost, Crozier, and Schn 2005, Flynn et al. 2006, Buckland et al. 2007, Chasco, Hilborn, and Punt 2007, Newman et al. 2009, Branch and Hilborn 2010). Run reconstruction uses maximum likelihood estimation of a product of probability density functions describing arrivals, escapement, harvest, and ages or it uses state-space models and Bayesian estimation via MCMC or related techniques. Run reconstruction methods have been widely applied for mixed stock fisheries in coastal fisheries although they have been applied to riverine settings as well (Eggers and Bernard 2011, Pritt et al. 2013).

There is no statistical literature on properties of estimators and confidence intervals for composition determined from direct identification. This paper provides a statistical evaluation of estimators derived via direct identification methods. We observe adult Chinook salmon and steelhead returning to Lower Granite Dam (LGD) on the Snake River 695 kilometers from the ocean. Returning adults enter the Columbia River from the Pacific Ocean and travel to the Snake River to spawn; they proceed through seven other dams (four Columbia River and three Snake River) before arriving at LGD. Fish arriving at LGD then disperse to tributaries throughout the Snake River basin to spawn. Counting and sampling returning adult salmon and steelhead at the dam provides direct data for calculating composition.

We examine the properties of estimators of composition and their confidence intervals derived from weightings of window counts, wild/hatchery data obtained from trap samples, and sex/age/genetic stock data collected on wild fish subsampled at the trap. One-at-a-time and simultaneous confidence intervals are derived via a combination of bootstrap sampling of both the wild/hatchery trap data set and the sex/age/genetic stock data set produced by sampling wild fish. We consider the window counts to be a census of fish passing the dam. Joint confidence intervals are found for sex, age, genetic stock, stock-sex, and stock-age. In earlier work (Steinhorst et al. 2010), we found that confidence intervals for small groups of fish like those formed by combinations of stock-sex and stock-age were too wide to be useful. Joint confidence intervals on such groups would be even wider.

It is useful to have one-at-a-time and joint confidence intervals for sex or age or genetic stock since, for the most part, numbers of wild females and males or numbers of wild fish by age or genetic stock are large enough to provide meaningful intervals. In some cases, there may be enough fish in categories formed by combinations of genetic stock-sex and genetic stock-age to make meaningful inferences, but this will depend on sample sizes.

In section 2, we describe the setting and the data collection and processing methods. In section 3, we present the estimators and methods for obtaining the bootstrap confidence intervals. In section 4, we give the results for salmon and steelhead runs spawning in 2011. Section 5 contains the results of simulations examining the properties of the estimators and confidence intervals. Our sampling goal is to know the true number of returning steelhead or Chinook within 10% with 90% confidence. If the distributions of our estimators are reasonably symmetric then the estimate will be close to the middle of the confidence interval. Since we are 90% confident that the true value is in the confidence interval, the difference between the estimator and truth will be less than 10% of the truth if half the confidence interval is less than 10% of the estimator.

2. DATA COLLECTION

This paper summarizes the abundance and composition of wild adult steelhead and spring/summer Chinook salmon returning to LGD during spawn year (SY) 2011. For steelhead, fish passing LGD during the summer and fall of 2010 and the spring of 2011 (07/01/10 – 06/30/11) comprise the run for SY2011. Spring/Summer Chinook arrive at the dam from April through August 17, 2011.

Adult salmon and steelhead returning from the ocean to the Snake River basin must pass LGD; adults enter a fish ladder bypass system to ascend past the dam. An observation window, located on the fish ladder, allows United States Army Corps of Engineers (COE) personnel to tally and record the number of fish for each species ascending the ladder daily. Snake River fisheries management agencies may assume daily fish counts are recorded without error and represent the total escapement to LGD. Video counts are used by COE in lieu of live window counts in November, December, and March. Live window count times were 0400 – 2000 Pacific Time, whereas video count times were 0600 – 1600. Most fish pass the window during the 10 to 16 daylight hours when counts are made (Cassinelli et al. 2012). Count data were downloaded from the COE website (http://www.nwp.usace.army.mil/Missions/Environment/Fish/Data.aspx). For Chinook salmon, the adult count (≥ 56 cm) was combined with the jack count (30-56 cm) to derive the total count on a daily basis. The steelhead count consists of all fish > 12” identified as *O. mykiss*. Daily counts were aggregated on a weekly basis. Early and late in the season weeks were combined if few fish were passing. (Tim, yes we should put the criterium here.)

The fish ladder bypass system also contains a trapping facility, located above the observation window, designed to intercept adult salmon and steelhead at a predetermined sampling rate (Harmon 2003; Ogden 2010, 2011; Schrader et al. 2012, 2013). Trap sampling rates are determined by a committee of co-managers in an attempt to achieve sample requirements for multiple projects and to balance fish handling concerns; sample rates are typically 10 – 20%. The sample rate determines how long a trap gate remains open four times per hour; the trap is operational 24 hours per day. Additional details on the adult trap can be found in Harmon (2003) and Steinhorst et al. (2010).

Trapped fish are anesthetized and examined to determine whether they are of hatchery or wild origin. All wild fish have an intact adipose fin (a small, fleshy fin on the back behind the dorsal fin and forward of the caudal fin) because they spend their entire life cycle in the natural environment. Most hatchery origin fish have a clipped adipose fin so that they can be identified for mark selective fisheries (i.e. recreational anglers may only keep fish with a clipped adipose fin). However, some hatchery fish may be released with an intact adipose fin to supplement natural populations or to bolster tribal harvest opportunities. Thus, fish with an intact adipose are further examined for a coded wire tag (CWT) or a passive integrated transponder (PIT) tag that were implanted into fish at the hatchery as juveniles. For adipose intact steelhead, hatchery origin may also be determined by the presence of dorsal and/or ventral fin erosion, which is assumed to occur only in hatchery-reared steelhead (Latremouille 2003). We also used the presence of a ventral fin clip to determine if an unclipped fish was of hatchery origin. For unclipped Chinook salmon, hatchery origin was determined solely by the presence of a CWT or ventral fin clip. After processing, all fish are returned to the adult fish ladder to resume their upstream migration.

Scale and tissue samples are taken from trapped fish determined to be wild to estimate sex, age, and genetic stock. Sex determination is not based on phenotypic characteristics observed at the LGD adult trap; rather sex is determined *post hoc* using a sex-specific allelic discrimination assay (Campbell et al. 2012) that is screened during genetic analysis (described below). The sex-determination assay for steelhead is 99.3% accurate and for Chinook salmon is 99.0% accurate based on comparisons with known-sex individuals (C. Steele and J. McCane, Pacific States Marine Fisheries Commission, personal communciation). Age is determined using visual examination of scale annuli (Schrader et al. 2013). Readers estimate both freshwater-age (time spent in freshwater prior to entering the ocean as juveniles) and saltwater-age (time spent in the ocean prior to upriver spawning migration as adults) for each fish. Ocean-age determination for steelhead is 98.8% accurate and for Chinook salmon is 98.1% accurate based on comparisons with known-age individuals. Currently, freshwater-age determination accuracy cannot be validated, but precision estimates based on the coefficient of variation between experienced readers were 11.9% for steelhead and 3.3% for Chinook (Schrader et al. 2013). Age data collected at LGD are used to assign returning adults back to specific brood years. Assignment of fish to brood year allows for cohort analysis and to estimate productivity and survival rates. In addition, escapement estimates by cohort are used to forecast run sizes in subsequent years. Forecasts are the basis for preliminary fisheries management in the Columbia River basin.

Scale samples were taken from above the lateral line and posterior to the dorsal fin. Samples were stored in coin envelopes for transport to the IDFG aging laboratory in Nampa, Idaho. Scales were mounted between two glass microscope slides and two technicians independently viewed each scale to assign ages without reference to fish length. If there was no age consensus among the readers, a third reader viewed the image and all readers collectively examined the image to resolve their differences before a final age was assigned. If a consensus age was not attained, the sample was excluded from further age-specific analyses.

The genetic stock of each fish was estimated using individual assignment (IA), a type of genetic stock identification (GSI; Pella and Milner 1987, Shaklee et al. 1999), using single nucleotide polymorphism (SNP) genetic markers. IA allocates each fish to the population and genetic stock in which the probability of its genotype occurring is the greatest. Two sets of data are required for IA: 1) allele frequency estimates from multi-locus genotypes obtained from samples taken from all potentially contributing stocks (the baseline), and 2) corresponding multi-locus genotype data from unknown origin individuals whose stock is to be estimated (Koljonen et al. 2005). Ackerman et al. (2014) present the Snake River SNP baselines for both steelhead and Chinook salmon used for this study; unknown stock fish at LGD were genotyped using the same SNP loci presented in that study. Each individual’s population was estimated and then summed into genetic stock estimates (allocate-sum procedure; Wood et al. 1987). Genetic stocks (reporting groups) are assemblages of reference (baseline) populations grouped primarily by genetic and geographic similarities and secondarily by political boundaries and/or management units (Ackerman et al. 2011). IA was performed using the Bayesian version of the program gsi\_sim (Anderson et al. 2008, Anderson 2010). The Bayesian gsi\_sim uses Markov chain Monte Carlo (MCMC) to computer posterior probabilities of stock membership conditional on the allele frequencies estimated from the baseline. The likelihood that a fish originates from a stock is computed using the compound Dirichlet-multinomial formulation of Rannala and Mountain (1997) conditional on the baseline samples and these likelihoods remain fixed throughout the MCMC simulation. To perform the MCMC, gsi\_sim uses a Gibbs sampler (Casella and George 1992) in which alternately: 1) the stock assignments of the fish in the mixture are updated as a multinomial draw from their posterior probabilities given the current estimate of the stock proportions and the stock-likelihoods of the fish; and 2) the stock proportions are updated as a draw from a Dirichlet distribution given a unit-information prior and the current values of the stock assignments of all the fish in the mixture.

Ackerman et al. (2014) defined the genetic stocks used for IA at LGD. Ten genetic stocks were defined for steelhead. Genetic stocks include: 1) UPSALM: the upper Salmon River; 2) MFSALM: Middle Fork Salmon River (including Chamberlain and Bargamin creeks); 3) SFSALM: South Fork Salmon River; 4) LOSALM: lower Salmon River; 5) UPCLWR: upper Clearwater River (Lochsa and Selway rivers); 6) SFCLWR: South Fork Clearwater River (including Clear Creek); 7) LOCLWR: lower Clearwater River; 8) IMNAHA: Imnaha River; 9) GRROND: Grande Ronde River; and 10) LSNAKE: Tucannon River, Asotin Creek and tributaries to the Snake River downstream of the Clearwater River confluence.

Seven genetic stocks were defined for Chinook salmon. Genetic stocks include: 1) UPSALM: upper Salmon River; 2) MFSALM: Middle Fork Salmon River; 3) CHMBLN: Chamberlain Creek; 4) SFSALM: South Fork Salmon River; 5) HELLSC: an aggregate stock that includes the Little Salmon, Clearwater, Grande Ronde, and Imnaha rivers and small tributaries of the Lower Snake River; 6) TUCANO: Tucannon River, and 7) FALL: Snake River fall Chinook salmon. The FALL genetic stock represents a separate lineage of Chinook salmon in the Snake River basin and is managed as a separate species under the ESA. Fall Chinook are genetically highly differentiated from spring/summer Chinook salmon and can be identified using IA with 100% accuracy (Ackerman et al. 2014).

Tissue samples for DNA extraction and SNP genotyping were taken from a small clip of the anal fin. Tissues were stored in a vial with 200-proof nondenatured ethyl alcohol for transport to the IDFG genetics laboratory in Eagle, Idaho. Genomic DNA extraction and amplification and SNP genotyping using multiplex 5’-nuclease reactions follow methods described in Seeb et al. (2009). DNA was extracted using nexttecTM Genomic DNA Isolation Kits from XpressBio (Thurmont, Maryland) or QIAGEN DNeasy Tissue Kits (Valencia, California). Ackerman et al. (2014) fully describes DNA amplification and SNP genotyping protocols; SNP genotyping was performed using the Fluidigm EP1TM platform. All adults queued for genotyping were screened at 191 SNPs and the sex-specific allelic discrimination assay (Campbell et al. 2012). Samples were processed at either the IDFG genetics laboratory in Eagle, Idaho, or the Columbia River Inter-Tribal Fish Commission genetics laboratory in Hagerman, Idaho.

It is important to note that due to the high accuracy of sex and age estimation, we were able to assume that sex and age were determined without error. However, error exists in the determination of genetic stock via IA. The magnitude of that error varies by genetic stock and is related to the relative genetic distinctness/similarity among stocks being estimated. In the simulations section of this paper, any uncertainty in the sex and age simulations is solely due to sample size and bias. For any simulations involving stock, we evaluate both the uncertainty that is due to sample size and bias plus uncertainty that is due to error in the assignment of genetic stock from IA.

The above sampling design produces three datasets: 1) a census of fish numbers returning to the dam (window counts), 2) a hatchery/wild dataset for all trapped fish, and 3) a dataset containing sex, age, and genetic stock for a sample of wild fish that were trapped. These three data sets are used to produce the size of the run and estimates and confidence intervals for numbers of fish by sex or age or genetic stock.

3. ESTIMATES AND CONFIDENCE INTERVALS

The proportions of wild and hatchery salmon or steelhead changes over the season, but within a week are relatively constant. Given weekly counts* ,* the number of wild salmon or steelhead is estimated as



where are estimates of proportion wild by week from the trap data. Since the window counts are assumed to be fixed, we find a confidence interval for the number of wild fish using a parametric bootstrap to produce B bootstrap sets of  yielding . That is, for week h we assume the bootstrap number of wild fish has a binomial distribution,  where  is the number of fish trapped for that week and . The and percentiles of give us the  confidence interval for the true number of wild Chinook or steelhead passing Lower Granite Dam for the year.

Define . Then we have as the conditional probabilities for wild females and males for weeks 1,…,*n.* Similarly for A ages, we have conditional probabilities .For g stocks, we have conditional probabilities . Given estimates of these probabilities from wild fish examined in the trap, we obtain





##### and



and



That is, the number of wild females or males is a weighted sum of the weekly window counts where the weights are estimates of the probability of being wild and female (or male). Likewise for ages 1,…,*A* or genetic stocks 1, . . . , *g ,* the estimate is a weighted sum of the weekly counts with weights that are an estimate of the probability of being wild of that age or genetic stock.

To find confidence intervals for these estimates we have to account for the variability of both the trap data and the wild fish sample data. We do this by extending the parametric bootstrap process used above for obtaining the confidence interval for number wild by adding a conditional parametric bootstrap based on sex, age, and genetic stock data from wild fish in the trap.

For sex define pseudoreplicates using



where is the number of wild fish sexed. Bootstrap values for the number of wild females,  follow from  *.* The percentile bootstrap confidence interval for true number of wild females,  is given by finding the and  percentiles. Similarly, we find a bootstrap confidence interval for the number of wild males. Changing the binomial above to a multinomial, we generate B sets of  to obtain bootstrap confidence intervals for the true number of wild fish of ages 1,...,A. We follow the same procedure for genetic stocks 1,... ,g.

Simultaneous confidence intervals for the number of females and males is found by expanding the rectangle formed by  and  until  of all of the pairs are inside the expanded rectangle (REF here). Likewise simultaneous confidence intervals for ages are found by expanding the A dimensional hypercube formed from the one-at-a-time intervals until  of the B A-tuples, , are inside of the expanded hypercube. The same expansion leads to simultaneous confidence intervals for genetic stock.

4. RESULTS FOR SPAWN YEAR 2011

We consider two examples to illustrate the methods outlined in section 3 - spring/summer Chinook and steelhead returning to spawn in 2011. This provides two differing examples; there were slightly less than 135,000 Chinook returning to LGD and over 200,000 steelhead. Chinook salmon start arriving at LGD in April and the run continues until August 17th which is operationally defined as the end of the spring/summer run and the beginning of the fall run. Fall run Chinook salmon are managed as a separate species under the ESA. The steelhead run arrives in two waves. The majority (94%) arrived in 2010 from July to November. The rest arrived in 2011 from March through June.

There were 134,594 Chinook counted at the observation window at the dam. Of those, 26,673 were estimated to be wild (19.8%, Table 1). Based on B=5000 bootstrap iterations, we obtained a 90% confidence interval of (25937,27400). We found 90% one-at-a-time and simultaneous confidence intervals on the numbers of females and males, on numbers by age (brood year), and on genetic stock. The simultaneous confidence intervals were found separately for sex, age, and stock.

[Table 1 about here.]

Note that one of the genetic stocks identified is FALL Chinook (4.2%) which are managed as a different stock/species. If more than 1,129 spring/summer Chinook arrived after August 18th, then our window count is an underestimate of spring/summer Chinook salmon escapement and vice-versa. There are two sexes, five ages, and seven genetic stocks. The simultaneous confidence intervals are proportionally wider for age and stock than for sex. Columns P1 and Psim give half the width of the confidence interval as a percentage of the estimate. If the estimate is near the center of the confidence interval then we are 90% confident that we know the truth within this percentage of the estimate. For total wild, the truth should be within 2.7% of the estimate. When the groups are quite small like BY05, BY09, and the TUCANO stock, then the truth might be as much as 35-50% away from the estimate. Note that the TUCANO stock (Tucannon River) is actually located below LGD; the TUCANO estimate represents fish that strayed above LGD or ascended LGD and fell back to the Tucannon River later. If you compare the P1 and Psim columns, you see that the widths of the simultaneous confidence intervals for female and male are not markedly wider than the one-at-a-time intervals. For age and genetic stock the simultaneous confidence intervals can be double the width of the one-at-a-time confidence intervals.

There were 208,296 steelhead returning during SY2011. Of these, we estimate that 44,452 were wild (21.3%, Table 2). The 90% confidence interval is (43454,45473). In addition to female and male, there are five ages, and ten genetic stocks. Confidence intervals for total wild, sex, BY05, BY06, BY07, GRROND, LSNAKE, and UPSALM meet our 10% goal for confidence interval width. BY04 and BY08 are not well estimated. The percent half widths of the other genetic stocks fall in the range 10.4 to 15.2%.

[Table 2 about here.]

For Chinook, there were nearly twice as many males as females. For steelhead, the reverse was true. These sex ratios vary annually, but the ratios seen for both species in 2011 are typical. For both Chinook and steelhead the middle age groups had more fish returning than the younger and older age groups. For Chinook, the HELLSC stock (Little Salmon, Clearwater, Grande Ronde, and Imnaha rivers and small tributaries of the Lower Snake River) was more than twice as abundant as any other stock. For stocks within the Salmon River, CHMBLN produced 616 spring/summer Chinook summer and the remaining 3 (UPSALM, MFSALM, SFSALM) all produced between 3,800 – 4,800 (Table 1). For steelhead the stocks are more evenly represented although the GRROND stock has almost four times the number as the SFSALM stock (Table 2). Note that the LSNAKE stock includes some populations below LGD (Tucannon River and small tributaries to the lower Snake River).

5. SIMULATIONS

We used a parametric bootstrap for this problem because the variables in both the trap data set and the wild data set follow a binomial or multinomial distribution. Using the observed weekly proportions, we generated bootstrap proportions for both the trap hatchery/wild and wild sex, age, and genetic stock weekly proportions. We then combined these bootstrap samples with the window counts to produce estimates and confidence intervals. While these estimators and confidence intervals are intuitive, we do not know their statistical properties.

We set up two simulations - one similar to the spring/summer Chinook scenario and one similar to the steelhead scenario. We established parameter values for all binomial and multinomial distributions that are similar to weekly estimates obtained from the data sets described above. We also set sampling rates for the trap (PTrp) and wild subsampling rates (PWhandled) similar to those used in practice at LGD.

For the Chinook simulation, we set the run size as 135,000 (Table 3). For our known population, 27,243 were wild (20%). Of those 8,867 were female (32.5%) and 18,375 were male (67.4%). The numbers of wild fish of each age (brood year) are found by multiplying the number wild (PopWild) column by the corresponding age column proportions (BY05...BY09). Summing those wild numbers by age over strata (weeks) gives the true numbers of fish of each age (BY05 = 184, BY06 = 8,271, BY07 = 14,660, BY08 = 3,975, BY09 = 153). Similarly, the numbers of wild fish of each genetic stock are found by multiplying the number of wild (PopWild) column by the corresponding genetic stock proportion columns. Summing those wild numbers by stock over strata gives the true numbers of fish of each genetic stock (UPSALM = 4,833, MFSALM = 4,519, CHMBLN = 623, SFSALM = 3,944, HELLSC = 11,937, TUCANO = 272, FALL = 1,114). Note that the trap rate was not constant over the season; PTrp runs from 7% to 11% (Table 3). Of the wild fish caught in the trap, sex and age was determined and stock was estimated for about three-fourths of them; PWhandled varies between 68% and 76%.

[Table 3 about here.]

We simulated 500 data sets as follows. Simulate the number of fish trapped each week by generating 14 binomial samples (weeks) with number of binomial trials equal to SimPop and probabilities PTrp. Simulate the number of trapped fish that are wild by generating 14 binomial samples with number of trials equal to the simulated number of trapped fish and with probabilities equal to PWild. The remaining trapped fish are hatchery. From these numbers, we can generate a simulated data set for trapped fish with 2 columns: week and wild/hatchery. The length of this data set is the sum of the numbers of wild fish trapped for the 14 weeks.

We can now calculate the random number of wild fish sexed, aged, and genotyped to estimate stock for each week by multiplying the random number of wild fish trapped by PWhandled. These 14 numbers are the number of binomial or multinomial trials for sex or age or genetic stock, *mh.* For example, we can find the random number of wild/handled fish that are female by generating binomial trials of size *mh* with probabilities Pfemale. The remainder each week are male. Knowing the random number of wild females and males each week, we can put together the simulated data set for sexed fish, say, by forming a data set with columns equal to stratum and female/male. The length of this data set is the sum of the 14 numbers of handled fish, .

Given the numbers of fish arriving at the dam each week, some 7 to 11 percent of them will be trapped (Table 3). The trapping data set will have about 13,500 lines (10% of 135,000). Depending on week ten to fifty-five percent of these will be wild and approximately 75% of these will be handled (Table 3) so the sex or age or genetic stock data set will be about 2,500 long.

Note that the simulated genetic stock data set represents the ‘known’ genetic stock of each fish and assumes that genetic stock can be determined via IA without error. However, for each simulated known stock fish we want to simulate SNP genotype data and then estimate an ‘inferred’ genetic stock using gsi\_sim. Doing so allows us to evaluate both uncertainty in trap sampling and uncertainty in genetic assignments. To do this…

[Table 4 about here.]

Now we have three random data sets: 1) random numbers of fish arriving each week, 2) a randomly generated trap data set with a random number of wild and hatchery fish, and 3) a randomly generated wild fish data set with randomly generated numbers of female/male or fish of various ages or fish of various stocks including both the known and inferred genetic stock of each fish. We use the window counts and the trap and wild data sets to obtain estimates and confidence intervals for that iteration. We save the estimates and confidence intervals for subsequent analysis. We used B=500 bootstrap iterations for finding the confidence intervals. After 500 simulation iterations, we have 500 estimates of total wild, female/male wild, wild by age, and wild by both known and inferred genetic stock. We also have 500 one-at-a-time and simultaneous confidence intervals for each estimate. Since we know the true number of wild fish and numbers of females/males and fish of different ages and different stocks, we compute the bias as the mean of the estimates minus the truth. We also compute the variances of the estimates and the standard errors.

We compute the coverage of any confidence interval by tallying the number of times the true population number falls inside the confidence interval. We get the simultaneous coverage by tallying the number of times the true population numbers fall within the expanded hypercube. For example, we know the true number of females (8867) and the true number of males (18375). The joint coverage is the proportion of times that the simultaneous female confidence intervals contain 8867 AND the simultaneous male confidence intervals contain 18375.

For simulated Chinook in 2011, we find that the estimates of total wild, number of females, and number of males are essentially unbiased (Table 5). The coverages of the one-at-a-time confidence intervals are very close to the nominal 90%. For females and males, our goal of estimating true numbers within 10% with 90% confidence was met (column P1). The expected half widths as a percentage of the truth are 5.8% and 3.6% respectively. The coverage of the joint confidence intervals for numbers of females and males is 93%. For ages, there is some bias in estimating the least abundant ages BY05 (3.50%) and BY09 (-1.97%) and the coverage of the one-at-a-time confidence interval for BY09 is slightly less than nominal (85.6%). The one-at-a-time confidence intervals for brood years BY06, BY07, and BY08 meet our sampling goals (column P1); the half widths as a percentage of the truth are 8.9% or smaller. For brood years BY05 and BY09, the expected half widths as a percentage of the truth are over 40%. The joint coverage for the age simultaneous confidence intervals is slightly below nominal (87%). The simultaneous confidence intervals are 1.2 to 3.3 times the corresponding one-at-a-time confidence intervals.

The bias across genetic stocks ranged from 0.1% (CHMBLN) to -5.7% (TUCANO) when accounting for uncertainty in both trap sampling and IA. The one-at-a-time confidence interval coverages were close to or achieved nominal for four of the seven stocks (CHMBLN, FALL, HELLSC, TUCANO). The remaining three stocks achieved confidence interval coverages of 75% or greater; MFSALM (82.8%), SFSALM (78.0%), and UPSALM (75.8%). Four of the percent half confidence criteria were 9.0% or less. The other three were 23.4% (CHMBLN), 15.9% (FALL), and 35.0% (TUCANO). The joint confidence was 0.93. The ratios of the one-at-a-time and simultaneous expected widths ranged from 1.2 to 2.2.

[Table 5 about here.]

The steelhead simulation was based on 200,000 returning steelhead (Table 6). Across stratum, the proportion of fish trapped (PTrp) ranged from 4 to 14%. Of the total return, 43756 (22%) are wild; Pwild range from 20 to 50% by stratum. Of the wild fish caught in the trap, 35 to 100% were handled. Of the wild fish, 28972 are female (66%). For ages, 2% are brood year 04; 24% are BY05; 48% are BY06; 24% are BY07; and 2% are BY08.

[Table 6 about here.]

Ten genetic stocks are represented in the steelhead data (Table 7). The true numbers across genetic stocks range from 1551 (3.5%, LOSALM) to 9607 (22.0%, LSNAKE).

We ran simulations for steelhead using the same protocol as for spring/summer Chinook (Table 8). When we computed one-at-a-time and simultaneous confidence intervals for sex or age or genetic stock, we obtained results similar to the Chinook simulations. For sex, the estimators are unbiased. The coverage of one-at-a-time confidence intervals for females and males were 89.4% and 88%, respectively. The expected half widths as a percentage of the truth were 3.4% and 5.4% respectively. The joint coverage for females and males was 90.2%. The expected widths for the joint confidence intervals for females and males were 1.3 and 1.2 times the expected widths for the one-at-a-time confidence intervals.

[Table 7 about here.]

The one-at-a-time coverages for BY04, BY05, BY06, BY07, and BY08 were all very near the nominal 90%. The expected half widths as a percentage of the truth were less than 10% for BY05, BY06, and BY07, but were 24.5% and 23.8% for BY04 and BY08, respectively. The joint coverage for ages was 90%. The expected widths of the simultaneous confidence intervals for ages were 1.2 to 2.2 times the expected widths of the one-at-a-time confidence intervals. We do a very good job of estimating numbers of females and males and a modestly good job of estimating numbers by age.

[Table 8 about here.]

The bias across genetic stocks ranged from 1.7% (UPCLWR) to -19.5% (LOSALM) when accounting for uncertainty in both trap sampling and IA. Six of the stocks had bias less than 5% (UPSALM, MFSALM, SFSALM, UPCLWR, SFCLWR, IMNAHA). For steelhead, only three stocks had one-at-a-time confidence interval coverages close to nominal; MFSALM (88.6%), SFSALM (86.4%), and UPCLWR (87.0%). All other stocks ranged from 27.2% (LSNAKE) to 82.0% (SFCLWR). Three of the 10 stocks met the percent half width confidence criteria of 10% or less (UPSALM, GRROND, LSNAKE); the remaining seven stocks were all less than 20%. The joint confidence for stocks was 0.xx. The ratios of the one-at-a-time and simultaneous expected widths ranged from 1.4 to 2.0.

Stock-sex and stock-age results for both Chinook and steelhead…

Our original goal was to find one-at-a-time and simultaneous confidence intervals for major groups of fish-sex, age, and genetic stock. We are also interested in sex-stock and age-stock confidence intervals.It is natural of course to want confidence intervals for numbers of fish for combinations of sex, age, and genetic stock. For Chinook, there are 10 sex-age combinations; 14 sex-stock combinations; and 35 age-stock combinations. If all combinations occur, there would be 2 \* 5 \* 7 = 70 sex-age-stock groups of fish. For steelhead there are 10 sex-age combinations; 20 sex-stock combinations; and 50 age-stock combinations. If all combinations occur, there would be 2 \* 5 \* 10 = 100 sex-age-stock groups of fish. Some of these groups have a fair number of fish and finding confidence intervals for them is reasonable. Some groups have so few fish it would be futile to try to find confidence intervals. Trying to find simultaneous confidence intervals for a large number of groups of fish would be a fool's errand. In addition to the sex, age, and stock simulations reported above, we simulated estimates and one-at-a-time confidence intervals for sex-age, sex-stock, age-stock, and sex-age-stock for both Chinook and steelhead. For this simulation, we assume that sex, age, and stock are independent so that we can multiply the probabilities in Tables 3, 4, 6 and 7 to get the joint probabilities. The results were mixed.

For Chinook, the one-at-a-time confidence interval coverage is above 0.90 for all groups of size 50 or more. For groups smaller than 50 the coverages range from 0.02 to 0.92. For groups of 300 or more, the half confidence interval widths as a proportion of the truth range from 4.3% to 39%. For groups of sizes 50 to 299, the proportional widths range from 40% to 100%. For groups of less than 50 the proportional widths range from 100% to 170% (Figure 1).

[Figure 1 about here.]

The steelhead simulation involves 200,000 fish compared to the Chinook simulated population of 135,000. Subgroups are larger in general. One result is that all one-at-a-time confidence interval coverages are 0.45 or larger. For group sizes between 50 and 100, the coverages for Chinook were all above 0.9. For steelhead, half of these coverages are smaller-between 0.8 and 0.9. For steelhead, all of the coverages for groups less than 50 fall below 0.9. The proportional half widths follow a similar pattern to those seen for Chinook except that the proportions are 144 or less rather than 170 or less (Figure 2).

[Figure 2 about here.]

Both Chinook and steelhead groups of 300 or more have good coverages and reasonably narrow confidence intervals. For group sizes between 100 and 300, the coverages are 0.9 or better, but the confidence intervals are somewhat wider. For groups below 100, the coverages may or may not be close to nominal and the confidence intervals are wide.

6. DISCUSSION

Steinhorst et al. (2010) recommended that group sizes should be at least 300 to be confident that you can estimate the true numbers within 10% with 90% confidence. In the figures above, we see that if the group size is 100 or less the coverage may be low and the expected confidence interval wide. In this study, we also found that coverage could be low and/or expected confidence intervals could be wide when a group is smaller (Kirk, check this out) than other groups in the set (see Table 8 where female coverage is low and brood years BY04 and BY08 have low coverage and wide confidence intervals even though all groups are bigger than 100).

Our goal of knowing the true group size within 10% with 90% confidence may be too restrictive. If there are only a few fish in a category, maybe we would be happy with a more lenient rule. For example, if our estimate is 50 and the confidence interval is (20,80), then the percent half width is 60% but knowing that the true number is between 20 and 80 might be sufficient.

When the numbers for a group are relatively small, then we worry about the distribution of pseudovalues being skewed. If so, the estimate may not be near the middle of the confidence interval. For the Chinook data set, the estimate of number of fish from BY05 is 148. We captured the 5000 (Kirk, check this out) pseudovalues for this case and looked at the distribution (Figure 3). Even though this group (and BY09) are much smaller than the other groups, the distribution of pseudovalues is pretty symmetric. The midpoint of the confidence interval (68,240) is 154. The problem, then, is not asymmetry, but rather coverage and/or confidence interval width.

[Figure 3 about here.]

Overall, our estimators and confidence intervals have good properties for major groups of fish. When the absolute number of fish in a group is low or relatively low compared to other groups, then there may be a problem with coverage and/or confidence interval width. Our joint confidence intervals for females and males are quite good. In our simulations, age and stock had confidence interval coverages of 0.856 or higher, but in some cases the confidence intervals were wide. When we try to find confidence intervals for combinations of sex, age, and genetic stock, the results are mixed depending on the size of the groups formed.

Our results have implications for how fish populations are monitored. If the interest is on the relative composition of the total (Tim, I am lost here), then most sampling programs will yield sufficient results. However, weak stocks are frequently a problem in conservation and fisheries management (CITE). We should make some positive statements about how to sample and to analyze monitoring data. Obviously there is a tradeoff between sample size and number of subdivisions that can be supported. Previous work (Gerritsen) shows this but the criteria for success is on overall (average) coverage. Thompson 1987 found that a sample size of 510 should suffice under a worst case scenario for alpha=0.05 (even proportions among groups but number of groups doesn’t matter). Our results show something else. What can we recommend?

Increasing sample sizes should lead to better results. For Chinook, the trap rate averages 10% and the handling rate is about 75%. For steelhead, the trap rate also averages 10% and the handling rate is a little over 50%. We reran Chinook and steelhead simulations increasing trap rates to 20% and the handling rate to 100%. This doubles the number trapped in both cases. In the case of chinook, the number of fish for which sex, age, and stock is determined is 2 2/3 times the original simulation. For steelhead, the number of fish for which sex, age, and stock is determined is 4 times the original simulation. It is likely that that level of sampling would swamp the trapping crew, but we wanted to see the effects of increasing the sample sizes to this level. For both species the percent half widths relative to true population sizes were reduced in proportion to the reciprocal of the square root of the increase in sample size-- for Chinook and  for steelhead. That is, the Chinook confidence intervals were 63% of their original size and the steelhead confidence intervals were half their original size. When the increased sampling leads to sample sizes of 300 or more, we meet our sampling goals. In other cases, sample sizes remain too small.

Although our case study is characterized by a high degree of sampling control (near census with systematic compositional samples), there are lessons for other fisheries applications. Certainly SY2011 had more control than other years at Lower Granite Dam (cite some Schrader reports) in that the run was as expected and there were no unexpected gaps in sampling. Frequently, sampling rate changes due to logistical concerns (e.g., more fish than expected can overwhelm trap and processing capacity) or environmental issues such as high summer temperatures. These types of problems will be greater in other cases where total abundance estimates have considerable variance or the sampling rate of the population for compositional data is small or unknown. The value of our case study is that it provides a best-case scenario to illustrate some basic statistical issues.

In short, the two data set bootstrap method for finding confidence intervals worked well. Our method for estimating numbers of steelhead and spring/summer Chinook in compositional groups provides unbiased estimators and reasonably narrow confidence intervals if the group size is 300 or more. If the group size is 100 or more the confidence interval coverage is generally close to the nominal value (95%), but the confidence intervals may be wide. Estimators and confidence intervals for groups of less than 100 may not have good statistical properties.

REFERENCES

Ackerman, M. W., N. Vu, J. McCane, C. A. Steele, M. R. Campbell, A. P. Matala, J. E. Hess, and S. R. Narum. 2014. Chinook and Steelhead Genotyping for Genetic Stock Identification at Lower Granite Dam. Idaho Department of Fish and Game Report 14-01. Annual Report, Bonneville Power Administration Project 2010-026-00. Available at: <https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res14-01Ackerman2014%20Chinook%20and%20Steelhead%20Genotyping%20for%20GSI%20at%20LGR.pdf>

Ackerman, M. W., C. Habicht, and L. W. Seeb. 2011. Single-nucleotide polymorphisms (SNPs) under diversifying selection provide increased accuracy and precision in mixed-stock analyses of sockeye salmon from the Copper River, Alaska. Transactions of the American Fisheries Society. 140(3):865-881. DOI: 10.1080/00028487.2011.588137

Anderson, E. C. 2010. Assessing the power of informative subsets of loci for population assignment: standard methods are upwardly biased. Molecular Ecology Resources 10(4):701-710.

Anderson, E. C., R. S. Waples, and S. T. Kalinowski. 2008. An improved method for predicting the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65:1475-1486.

Busby, P. J., T. C. Wainwright, G. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Wauneta, and I. V. Lagomarsino. 1996. Status review of West Coast steelhead from Washington, Idaho, Oregon, and California. NOAA Technical Memorandum NMFS-NWFSC-27.

Campbell, M. R., C. C. Kozfkay, T. Copeland, W. C. Schrader, M. W. Ackerman, and S. R. Narum. 2012. Estimating Abundance and Life History Characteristics of Threatened Wild Snake River Steelhead Stocks by Using Genetic Stock Identification. Transactions of the American Fisheries Society. 141(5):1310-1327. DOI: 10.1080/00028487.2012.690816

Casella, G. and E. I . George. Explaining the Gibbs Sampler. The American Statistician. 46(3):167-174.

Cassinelli, J., S. Rosenberger, and F. Bohlen. 2012. 2011 Calendar Year Hatchery Chinook Salmon Report: IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho. Idaho Department of Fish and Game Report Number 12-02. Available at: <https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res12-02Cassinelli2011%20Calendar%20Year%20Hatchery%20Chinook.pdf>

Harmon, J. R. 2003. A trap for handling adult anadromous salmonids at Lower Granite Dam on the Snake River, Washington. North American Journal of Fisheries Management 23:989-992.

Hilborn, R., and Walters, C. J. (1992), *Quantitative Fisheries Stock Assessment: Choice, Dynamics, and Uncertainty*, New York: Chapman and Hall.

Koljonen, M. L., J. J. Pella, and M. Masuda. 2005. Classical individual assignments versus mixture modeling to estimate stock proportions in Atlantic salmon (*Salmo salar*) catches from DNA microsatellite data. Canadian Journal of Fisheries and Aquatic Sciences 62:2143-2158.

Latremouille, D. N. 2003. Fin erosion in aquaculture and natural environments. Reviews in Fisheries Science 11:315-335.

McDowall, R. M. (1999) , "Tim I need the ref here," *I dont know,* 99, 1-999.

Ogden, D. A. 2012. Operation of the Lower Granite Dam adult trap. National Marine Fisheries Service. Annual report 2010, BPA project 2005-002-00.

Ogden, D. A. 2011. Operation of the Lower Granite Dam adult trap. National Marine Fisheries Service. Annual report 2009, BPA project 2005-002-00.

Pella, J. J. and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. Pages 274-276 *in* N. Ryman and F. Utter, editors. Population genetics and fisheries management. University of Washington Press, Seattle.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the USA 94:9197-9201.

Raymond, H. L. 1988. Effects of hydroelectric development and fisheries enhancement on spring and summer Chinook salmon and steelhead in the Columbia River basin. North American Journal of Fisheries Management 8:1-24.

Schrader, W. C., M. P. Corsi, P. Kennedy, M. W. Ackerman, M. R. Campbell, K. K. Wright, and T. Copeland. 2013. Wild adult steelhead and Chinook salmon abundance and composition at Lower Granite Dam, spawn year 2011. Idaho Department of Fish and Game Report 13-15. Annual report 2011, BPA Projects 1990-055-00, 1991-073-00, 2010-026-00. Available at: https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res13-15Schrader2013%20Wild%20Adult%20Steelhead%20and%20Chinook%20Salmon%20Abundance.pdf

Schrader, W. C. T. Copeland, P. Kennedy, M. W. Ackerman, K. K. Wright, and M. R. Campbell. 2012. Wild adult steelhead and Chinook salmon abundance and composition at Lower Granite Dam, spawn year 2010. Idaho Department of Fish and Game Report 12-16. Annual report 2010, BPA projects 1990-055-00, 1991-073-00, 2010-026-00. Available at: https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res12-16Schrader2012%20Wild%20adult%20steelhead%20and%20Chinook%20salmon%20abundance.pdf

Seeb, J. E., C. E. Pascal, R. Ramakrishnan, and L. W. Seeb. 2009. SNP genotyping by the 5’-nuclease reaction: advances in high-throughput genotyping with nonmodel organisms. Pages 277-292 in A. Komar, editor. Methods in molecular biology: single-nucleotide polymorphisms, 2nd edition. Humana Press, Totowa, New Jersey.

Shaklee, J. B., T. D. Beacham, L. Seeb, and B. A. White. 1999. Managing fisheries using genetic data: case studies from four species of Pacific salmon. Fisheries Research 43:45-78.

Steinhorst, K., D. Milks, G. P. Naughton, M. Schuck, and B. Arnsberg. 2010. Use of Statistical Bootstrapping to Calculate Confidence Intervals for the Fall Chinook Salmon Run Reconstruction to Lower Granite Dam. Transactions of the American Fisheries Society 139:1792-1801. DOI: 10.1577/T09-200.1

Wood, C. C. S. McKinnell, T. J. Mulligan, and D. A. Fournier. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Canadian Journal of Fisheries and Aquatic Sciences 44:866-881.