

Genetic parentage analysis to evaluate reproductive success of spring Chinook salmon (*Oncorhynchus tshawytscha*) in Newsome Creek, Idaho

2013 Annual Report

(8-10-14)

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Background

The Clearwater River Subbasin historically supported large spawning populations of Chinook salmon (*Oncorhynchus tshawytscha*) that represented a significant cultural, economic and subsistence resource for the Nez Perce Tribe. Extirpation of spring Chinook salmon throughout the subbasin occurred following construction of the Lewiston Dam in 1927. However, spring Chinook salmon were reintroduced in Clearwater River tributaries beginning in 1971 via release of hatchery-reared juveniles originating from multiple out-of-basin stocks, including Carson Hatchery, and Rapid River Hatchery (see HGMP 2011). Following initiation of reintroduction efforts, brood stock have been comprised of hatchery and natural origin returns captured in-basin. Returning adult hatchery fish reared as juveniles in the Nez Perce Tribal Hatchery (NPTH) are coded wire tagged (CWT) and are designated as “supplementation”. Natural-origin or “naturalized” fish are not tagged and have no physical markings. Although naturalized, the current local populations in the Clearwater River are not considered wild because they were re-established from non-endemic stocks, and thus are not listed under the ESA (Waples et al. 1993; Narum et al. 2007).

The Nez Perce Tribal Hatchery was constructed to restore natural spawning Chinook salmon aggregates throughout the available habitat in the Clearwater River Subbasin. Releases of spring Chinook salmon occur in three populations including Newsome Creek, a tributary to the South Fork Clearwater River. In past years the broodstock for the Newsome Creek supplementation program included adult Chinook salmon captured at the Newsome Creek weir, plus additional fish that returned directly to the NPTH. In 2012, all adult spring Chinook salmon that returned to the Newsome Creek weir were passed above the weir for natural spawning, and 100% of the Newsome Creek broodstock was derived from swim-ins to NPTH. Specific long term goals for NPTH monitoring and evaluation throughout the Clearwater River Sub-basin are aimed at understanding genetic interactions between naturalized and hatchery-origin fish, trends in natural productivity, local demographic effects resulting from Chinook salmon supplementation, and sustainability of harvest (Backman et al. 2006).

Parentage analysis provides a means to directly and accurately evaluate natural productivity on the basis of several metrics including relative reproductive success (RRS) between different categories of fish (i.e. hatchery vs. natural origin). This report summarizes results of overall Chinook salmon productivity in Newsome Creek. The results herein specifically focus on RRS among naturalized fish and NPTH origin fish (e.g., those with a CWT); and to a lesser degree any strays (ad-clipped) originating from other releases in the subbasin. The primary long-term objective is to quantify the net demographic benefit of supplementation as a component of the natural spawning population. The overarching question is whether or not naturalized fish demonstrate a reproductive success rate that differs significantly from natural spawning hatchery-origin fish. If so, this may suggest an adaptive advantage among the naturalized population. In this and subsequent reports the potential effects of hatchery rearing on natural reproduction is evaluated at two stages: 1 - proportions of naturally produced juvenile and adult progeny between hatchery and natural origin parents (F1), 2 – reproductive success following adult-to-adult returns (F2) between the two population components. Additionally, these analyses aid in determining weir efficiency, and verification of both gender ID and origin ID for fish sampled at the weir and/or during spawning surveys.

Methods

All previous parentage analyses for Newsome Creek spring Chinook salmon were evaluated using genotypic data from a suite of 15 microsatellite (μ SAT) markers (2012 Annual Report; Matala et al. 2013). Beginning with the analyses performed in the current report year (2013) a suite of 192 single nucleotide polymorphism (SNP) loci were employed to evaluate parentage; this applies to collections of adults and juveniles from calendar year 2012. In addition, both CRITFC staff and Nez Perce fisheries managers agreed that genotyping of SNPs would be applied retroactively to Newsome Creek adults spring Chinook salmon sampled during brood years (BY) 2008-2011. Note that the switch to SNP panels did not apply to Newsome Creek juveniles sampled between calendar year 2009 and calendar year 2011. Therefore, the results of reproductive success for BY2008 through BY2009 that are summarized in the current report are contingent on the assignment results from previous reports (i.e. based on μ SATs). Parentage analysis relevant to BY2010 spans the transition in marker type, and therefore includes assignments of pre-smolts (fall 2011) based on μ SATs, and assignment of smolts (spring 2012) based on SNPs.

Biological tissue was sampled (fin clip or opercle punch) from all adult spring-run Chinook salmon trapped and passed at the Newsome Creek weir from brood year 2008 to 2012. Carcasses encountered during spawning ground surveys were also sampled unless too greatly decomposed. NPTH hatchery origin individuals were identified on the basis of CWT detection accompanied by no physical marks (adipose fin intact), while putative naturalized fish were identified by the absence of both CWT and physical marks. Other hatchery stocks introduced in the Clearwater River are presumably recognizable as “strays” on the basis of fin clip markings. Among all adult Chinook salmon sampled from BY2008 to BY2012, only 24 individuals were excluded from analysis, largely on the basis of missing genotypic data (i.e. zero score; Table 1). Outmigrating juveniles from calendar years 2009-2012 were sampled in the spring (smolts) or fall (pre-smolts or precocial parr) during screw trap operations in Newsome Creek.

Pedigree Analysis

Locus specifications and relevant laboratory protocols for PCR amplification and scoring of bi-allelic SNP loci are described in Hess et al. (2012), and Hess et al. (2013). In summary, one panel of 96 SNPs has been specifically compiled for parentage-based tagging (PBT) of Snake River hatcheries. The panel includes a sex determining marker (*Ots_SEXY3-1*) that will allow difficult field identifications to be verified or corrected. The PBT panel can also be used to verify origin of adult fish recorded at the Newsome Creek weir (i.e. natural, NPTH, or non-program hatchery fish). Field identifications at the weir are frequently complicated by tag loss and tag scanning errors, as well as the presence of unmarked hatchery fish (i.e. South Fork Clearwater supplementation stock). Minimizing bias from misclassification errors is vital for obtaining accurate parentage assignments and estimates of reproductive success relative to either spawner origin or spawner sex.

The program CERVUS version 3.0 (Marshall et al. 1998; Kalinowski et al. 2007) was used to conduct parentage analysis. The method requires two supporting statistics generated for likelihood based parentage procedure. First, allele frequency analysis was conducted in CERVUS to assess the resolving power (accuracy of assignment) for the suite of loci using the entire candidate parent dataset (adults sampled from 2008-2011). From the allele frequency

results, a combined non-exclusion rate was calculated. This statistic is defined as the probability that an unrelated candidate parent (or parent pair) would not be excluded in parentage assignments (i.e. non-parent counted as parent). The combined non-exclusion rate for “identity” is the probability that the genotypes between two randomly-chosen individuals do not differ. Second, an initial parentage simulation was performed, where the identity of the simulated (true) parent is “known” for each simulated offspring. The following simulation parameters were used: analysis type - “parent sexes unknown”, $n=5,000$ offspring, $n=250$ candidate parents, proportion of parents sampled = 0.85, proportion loci typed = 0.99, proportion loci mistyped = 0.01, and error rate in likelihood calculations = 0.01. Simulations provide an assignment confidence threshold or critical value of the log-likelihood statistic (LOD) so that the confidence of parentage assignments can be evaluated statistically. The LOD score is calculated as the natural log (\log_e) of the likelihood ratio between the most likely parent assignment and the next most likely assignment; the distribution of LOD for assigned non-parents vs. assigned true parents can be plotted from simulation results (Supplemental 1). Parentage assignments were evaluated using two levels of stringency to guard against false positive assignments (type-1 error); relaxed confidence (0.95) and strict confidence (0.99).

Following power analysis and simulation tests, CERVUS was used to perform parentage analysis by evaluating (matching) multilocus genotypes between Newsome Creek adults and juvenile offspring. Parentage was conducted without a gender designation (i.e., “unknown” parent sex), allowing males and females identified in the field to be paired with same sex partners in parentage analysis when those pairings proved to be the most likely based on statistical probability. For assigning pedigrees, the use of a full-exclusion approach is necessarily contingent on, or limited to zero mis-matched loci between parent and offspring, and is generally considered as the highest standard for providing accurate results. However, to account for genotyping error, all assigned parent pairs having two or fewer observed mismatches were given further consideration contingent on strict confidence criteria and LOD thresholds as outlined previously. Single parent assignments were accepted on the basis of two criteria: 1) zero mismatched loci observed at a strict confidence threshold for LOD (Supplemental 1), or 2) a single mismatching locus and a LOD valued exceeding the overlap in LOD distributions between true parent and non-parent.

Reproductive success (RS) was calculated as the ratio of the total number of sampled offspring to the total number of sampled candidate adults (or assigned parents). Relative reproductive success (RRS) is the ratio of RS for natural-origin spawners to hatchery-origin spawners (N_{RS}/H_{RS}) or vice versa (H_{RS}/N_{RS}). Both RS and RRS were calculated for each brood year independently (BY2008 to BY2010), and for each juvenile age category (pre-smolt, smolt, precocial). The overall temporal RRS was evaluated using a geometric mean. In addition, the RRS between the naturalized and hatchery population components was evaluated on the basis of two scenarios: 1) all sampled adults, and 2) assigned parents only. The latter scenario excludes all adult fish that were not contributors to overall reproductive success (i.e. zero assigned progeny). Internal tests of the parentage method were incorporated in order to estimate error and validate the accuracy of parentage assignment. Specifically, 2012 adult returns were included in the data set to evaluate parentage of 2012 juveniles, and 2010-2011 candidate adults were included in the data set to evaluate parentage of 2012 adult returns. Because these comparisons are anachronistic (e.g. indicative of age-0 or age-1 returning adults) the expectation was that no

parent/offspring relationships would be observed. Those that might occur represent potential biases in the accuracy of the analysis.

Results

Descriptive statistics and parentage simulation

The Microsoft Excel add-in “Microsatellite Toolkit” was used to identify a single pair of 2012 sampled adults with identical genotypes; the duplicate sample was a male sampled at the weir (identified as hatchery-origin) and subsequently during carcass surveys (identified as natural-origin). Six juvenile and five precocious parr samples were also sampled multiple times among calendar year 2012 collections. One individual from each duplicate pair was removed prior to evaluation of reproductive success. All other duplicate sampling issues were previously resolved and reported (Matala et al. 2013). The final number of candidate parents used in parentage analysis was pared from the number sampled after excluding samples with excess missing genotypic data (Table 1a).

Results of allele frequency analysis used to assess assignment accuracy revealed a non-exclusion rate for assignment of 0.00019 for the first parent (1 in 5,300 comparisons), $3.7E-00^9$ for the second parent (when the first is identified), and $7.1E-00^{15}$ for the parent pair. The observed combined non-exclusion probability for identity was $1.4E-00^{39}$. Based on parentage simulation, the critical LOD at a strict confidence level of 0.99% was LOD= 1.25 for single parent assignment, and LOD= 14.50 for a parent pair. In other words, the assignments observed among Newsome Creek samples would be considered to have 99% confidence (or better) when LOD exceeds these threshold values. Further, the simulated LOD distributions for single non-parent and single true parent show good separation. However, the distributions overlap between LOD= -2 and LOD= 13 in the respective tails of the distributions for single parent assignments and between LOD= 15 and LOD= 24 for parent pairs (Supplemental 1). The simulated mean LOD was 18.3 for true parent (single) and 47.4 for true parent pairs. Most single parent assignments and parent pair assignments were determined on the basis of full exclusion. All likelihood based assignments (accepted with one mismatched locus) necessarily required a LOD that both exceeded the 99% critical threshold, and was greater than the LOD distribution overlap between true parent vs. non-parent (e.g., LOD>14 for single parent assignments). Note that the assignment statistics discussed above pertain to SNP genotypes. Simulation thresholds and confidence criteria for assignments based on μ SAT genotypes are available in the 2012 report (Matala 2013).

Parentage analysis: field data verification and correction

Prior parentage analyses of Newsome Creek spring Chinook have indicated variable rates of error in field data entries that correspond with genetic sampling (Matala 2011). Gender identification discrepancies as well as weir efficiency calculations (based on carcass data), and origin ID discrepancies are a frequent occurrence in these types of studies. The switch from μ SAT to SNP markers in the current report has proven valuable for combating many of these issues. Among assigned parent/offspring trios, 70 parent pairs were identified as individuals of the same gender based on field ID, including 25 unique pairs (some pairs were assigned multiple offspring). In every instance the sex determining SNP (*Ots_SEXY3-1*) allowed gender discrepancies to be resolved. Overall, gender corrections were necessary for 111 individuals (field ID vs. genetic ID) among all adult fish sampled between BY2008 and BY2012, which

greatly improved confidence in relative reproductive success results. Similarly, the PBT panel of SNPs allowed verification of field origin ID for all adult fish. Note that the current version of the PBT baseline was initiated with hatchery brood stocks genotyped in BY2008, and is therefore only equipped to identify 3 year old fish in the BY2011 return (jacks) and both 3-4 year old fish in the BY2012 return. There were 68 total PBT assignments of adult Chinook sampled at the Newsome Creek weir, including six BY2008 PBT progeny (jacks) sampled in Newsome Creek during the BY2011 return. The remaining 62 assignments were from the BY2012 Newsome Creek return, including 53 BY2008 assignments (age 4 years) and 9 BY2009 assignments (8 jacks, one female). Of the 68 assignments, 57 had NPTH brood stock parents. Three other hatchery stocks including McCall Fish Hatchery accounted for the remainder of assignments. Smolts from these stocks (BY2009) were released into the South Fork Clearwater River in 2011 (appendix 1).

Assignment criteria for adult-to-adult parentage analysis were identical to those previously described for juvenile offspring. All adult fish sampled in BY2012 (n=138) were evaluated as potential offspring from BY2008 (n=59 adults) and BY2009 (n=39 adults). However, only n=70 adult fish from the 2012 return were field identified as putative natural origin (i.e. offspring of natural spawners). Results revealed parent-pair assignments for only 5 of 138 sampled adult offspring, including two hatchery-x-hatchery parent crosses and two natural-x-natural parent crosses. Additionally, there were 22 single parent assignments dominated by natural-origin parents (Table 5). Similar to results of PBT analysis, errors in field ID were revealed through adult-to-adult parentage assignments, where five “hatchery-origin” 2012 adults were assigned as progeny of fish passed above the weir in either BY2008 or BY2009. Lastly, note that two candidate adult Chinook salmon from the BY2011 return were assigned with high likelihood and zero mismatched loci as the parents of BY2012 adults. These latter assignments would be indicative of age-1 adult returns, and are not reasonable. Although there are several sources of potential error including genotyping error, the best explanation is that the 2011 putative parents and 2012 putative offspring are actually full siblings, each with common genotypes. Therefore, throughout these and previous analyses, some degree of false assignment error (likely minor) should be assumed on the basis of kinship.

The combined results from adult-to-adult assignments and PBT assignments highlight a primary concern for the ongoing RRS evaluation that will need to be closely monitored and used to adjust estimates going forward. Both methods revealed significant error rates in field identification of origins. There were a total of 138 Chinook salmon sampled in Newsome Creek during the BY2012 return. Of these, n=68 were identified as hatchery-origin, yet only n=39 were confirmed using the PBT baseline, leaving 29 hatchery individuals unaccounted for. Concordantly, single parent assignments in adult-to-adult results (Table 5) indicate that at least five individuals were falsely identified as hatchery origin (i.e. having natural spawning parents). Together these observations suggest that the actual number of hatchery-origin fish sampled at the weir was fewer than 68. Similarly, of 138 Chinook salmon sampled in Newsome Creek during the BY2012 return n=70 were identified as natural-origin. However, there were 23 confirmed PBT assignments (i.e. hatchery-origin fish) among the 70 putative natural-origin samples (33%). The collection summary (Table 1) used in RRS calculations reflects corrections based on the discrepancies outlined above.

Parentage analysis: relative reproductive success

In BY2008 greater reproductive success was observed for hatchery-origin parents in regard to pre-smolt production. Conversely, smolt production was markedly higher among natural-origin parents (Table 2a). The outcome was similar for male and female parents, and for either the total adult sample or among assigned parents exclusively. Reproductive success in BY2009 was more difficult to assess because only a single hatchery female was sampled (and genotyped), and she was not assigned as the parent of any sampled offspring (Table 2b). Nevertheless, in BY2009 natural-origin female reproductive success was high regarding numbers of pre-smolt offspring (10.92/sampled adult, and 32.75/parent). For smolt production in BY2009 the RS results were less definitive. Results for BY2010 were based on a relatively large sample of both natural and hatchery origin adults (and assigned parents). In BY2010 the RS results ranged broadly between offspring age categories and depending on adult origin. Interestingly, results also tended to be gender specific. For pre-smolt and precocial production the RS favored hatchery males and natural-origin females, but the opposite result was observed in regard to smolt production (Table 2c). The overall results of RRS (geomean across brood years) are in contrast to the BY2010 results. The RRS for smolt production favored natural-origin males ($N_{RS}/H_{RS}=1.32$), but the RRS for pre-smolt production favored natural-origin females ($N_{RS}/H_{RS}=1.71$). For male and female candidates combined there was no difference in reproductive success between hatchery-origin and natural-origin adults (Table 3). Although the reproductive success of stray hatchery adults could not be factored into results for the RRS for natural vs. hatchery origin specifically reported here, stray spawners were highly productive in BY2009 with an RS=12.7 for males.

The most frequently observed cross type among parent pair assignments was hatchery-x-hatchery; note that in BY2012 hatchery parents outnumbered natural origin parents by greater than three to one. The H-x-H and H-x-N crosses displayed higher pre-smolt production (68% and 60% respectively), while N-x-N crosses had greater observed smolt production (61%; Table 4). The distribution of assigned offspring per parent was variable for both hatchery origin and natural origin adults, and equally variable for males vs. females. For all categories the largest proportion of sampled adults were assigned zero sampled offspring (Figure 1). The maximum observed number of assigned offspring was 46 pre-smolts to a female natural-origin parent, and 33 pre-smolts to a male hatchery-origin parent. Juvenile size-at-age can be calculated based on migration time independent of a genetic parentage analysis (presmolts and precocial parr - fall, smolts - spring). As an alternate means, results from genetic parentage assignments may be useful as a means of comparing size distributions determined via other methods. Brood year assignments of outmigrating juveniles from calendar year 2012 were used to examine fork length distributions corresponding to season. The three juvenile age classes examined had overlapping distributions, but distinct mean lengths that increased with age (Figure 2). Corroborating results were observed when mean length was plotted against ordinal day of outmigration (Figure 3). Significantly increasing growth trajectories were observed for both presmolts and smolts throughout their respective outmigration periods.

Conclusions/Future directions

Studies have suggested that some natural populations are likely to experience unsustainable (deficient) population growth rates (Johnson et al. 2012). Conservation programs utilizing hatchery supplementation have shown evidence of comparable or greater reproductive success for the hatchery-origin population component compared to a natural-origin counterpart (Hess et

al. 2012). On the other hand, accumulating evidence indicates the potential for decreased fitness and low natural reproductive success that may result from hatchery rearing (Chilcote et al. 1986; Chilcote 2003; Kostow 2003; Kostow 2004; Araki et al. 2007; Araki et al. 2008). The latter category of observation may not be representative of what occurs following extirpation of populations in large regions such as the Clearwater River subbasin. In such cases, the reestablishment of naturally spawning populations (and initially all productivity) is derived from hatchery-origin fish; therefore, the population is “naturalized” rather than natural-origin. Interpreting differential rates of reproductive fitness or smolt-to-adult survival between naturalized fish and their hatchery reared counterpart may not be comparable to that of indigenous natural origin populations competing with a supplemented population component (e.g. as in a conservation hatchery program, See Araki et al. 2007). Presumably, in a reintroduction effort the naturalized population is comprised of the fish best adapted to specific novel environments. This report covers results from BY2008 through BY2010 for the spring Chinook salmon reintroduction/supplementation program in Newsome Creek. Reproductive success was presented from the perspective of hatchery-origin spawners (H_{RS}/N_{RS}) which is the conventional perspective of this type of study. In addition, results were viewed from the alternative perspective where reproductive success was standardized to the “naturalized” population (N_{RS}/H_{RS}). The latter corresponds with the assumption that a reintroduction effort (or recolonization) is successful once a self-sustained natural spawning population is established. To accelerate this process the hatchery population should be reproductively successful in the short-term, then leading to increasing RRS and higher abundances in the naturalized population.

Results of relative reproductive success indicate a fairly large degree of inter-annual variation. Overall, the RRS for each individual brood year (BY2008 – BY2010) tended to favor hatchery-origin parents for pre-smolt production and natural-origin parents for smolt production. However, there is some evidence to suggest these results may be gender specific. The mean RRS for the combined three brood years is not statistically different between hatchery or natural origin parents, nor between males vs. females (see Table 3; combined geomean). Given limited data for BY2009, and somewhat opposing results between BY2008 and BY 2010 (see Table 2a-c) it remains difficult to identify or confidently support a trend/s in reproductive success between the NPTH hatchery stock and the naturalized population in Newsome Creek. However, further monitoring should reveal whether or not observed rates of reproductive success at the juvenile stage will carry through to subsequent returning adult progeny. For example, higher reproductive success observed for natural-origin parents based on smolt production may suggest higher overwintering survival compared to hatchery-origin spawner. Over the course of this genetic monitoring study it will be important to demonstrate whether or not the natural-origin progeny of hatchery spawners show improved productivity relative to their parents (e.g. increasing abundance of spawners). In other words, are adaptive potentials maintained (or retained) in the natural environment as supplementation continues? Because natural-origin adult spawners, particularly those of hatchery descent (i.e. naturalization of the hatchery population) complete their entire life cycle in the in-stream environment it seems reasonable to assume they as a group represent the best adaptive potential for conditions in the watershed (or S. F. Clearwater subbasin). Continued adult-to-adult monitoring should help clarify this assumption.

When using parentage analysis for supplementation monitoring, several confounding factors are frequently encountered. For example, persistent difficulties in accurate gender and origin

identification will introduce significant error or bias in assessments of relative reproductive success. A substantial number of assignments in these analyses were observed with same sex parent pairs, but in every case the sex determining SNP marker accurately identified male from female. In previous evaluations (Matala 2011; Matala 2012) mixed origin (hatchery and natural) sibling groups were identified (erroneously) as progeny of hatchery spawned parents. Additionally, in previous reports as well as the current report, origin ID error was recorded from genetic parentage assignment of adult offspring (but also through PBT analysis). Previously such errors precluded the ability to estimate reproductive success specific to parent gender, leaving reasonable uncertainty in RRS between population components (naturalized vs. hatchery). Together, the information provided in this most recent report allowed ID errors to either be flagged for correction in future evaluations (e.g., parentage analyses of 2013-2014 juveniles based on 2012 adult returns), or to be corrected immediately before final RRS conclusions were reached. Aside from insufficient genotypic data for individuals whose DNA failed to amplify successfully, the numbers of sampled juveniles and accordingly the numbers of candidate parents that remained unassigned in parentage analysis (no parent/offspring match) was in large part a consequence of missed sampling opportunities (e.g., late weir installation, unsampled carcasses). Up until the current report year there had been no precocial parr samples identified, yet in 2012 there were 146 precocials sampled from screw trap captures. The extent of parentage contributions from this “parent” category will be evaluated in the next annual report once the 2013-2014 juvenile collections are genotyped.

Acknowledgements

We appreciate the efforts of NPT field crews who conducting on-site genetic sampling. Vanessa Morman (CRITFC laboratory technician) conducted sample processing and data collection. We are grateful for funding by the Bonneville Power Administration, particularly the project COTR Barbara Shields. Thanks also to Peter Galbreath for allocation of funding from a broad based Accords basin-wide reintroduction and recolonization project, and for study design assistance.

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Table 1a. Adult collection summary of spring Chinook salmon evaluated in parentage analyses. Brood years (BY) 2008-2010 pertain to parentage analysis of juvenile offspring. Brood years 2008 and 2009 represent the candidate parent pool pertaining to adult-to-adult parentage for returning adult offspring in BY2012. Values in parentheses indicate # of sampled adults that were paired from the data set.

Origin	Newsome Weir gender	brood year collection					Total
		2008	2009	2010	2011	2012	
Adult							
natural	(M)	23 (2)	11 (1)	13	23 (3)	29 (1)	108
natural	(F)	11	13 (1)	9 (3)	21 (1)	24	81
hatchery	(M)	20 (2)	7	69 (1)	30 (2)	47	163
hatchery	(F)	3	1	58 (6)	39 (1)	39	135
stray	(M)	0	7 (1)	1	0	---	8
stray	(F)	2	0	1	0	---	2
total sampled (n)		59	39	151	113	138	500
missing genotype (n)		1	1	1	6	0	9
duplicate genotypes (n)		3	2	9	1	1	16
Final count for analysis		55	36	141	106	137	475

Table 1b. Summary of juvenile Chinook salmon collections. Season is indicated below each age category, and BY was determined on the basis of season and year of screw trap capture. Brood years with incomplete sampling (*) were genotyped but not evaluated in this report.

juvenile	brood year	sampled		genotyped		assigned	
		year	(n)	(n)	(% sampled)	(n)	(% genotyped)
pre-smolt (fall)	2008	2009	50	50	1.00	22	0.44
	2009	2010	349	313	0.90	205	0.65
	2010	2011	496	496	1.00	378	0.76
	*2011	2012	898	856	0.95	---	---
smolt (spring)	2008	2010	50	48	0.96	35	0.73
	2009	2011	28	22	0.79	13	0.59
	2010	2012	296	292	0.99	248	0.85
	*2011	2013	---	---	---	---	---
precocial (fall)	2008	2010	0	---	---	---	---
	2009	2011	0	---	---	---	---
	2010	2012	145	140	0.97	75	0.54
	*2011	2013	---	---	---	---	---

Table 2a. Relative reproductive success (RRS) summary for brood year 2008 (i.e. the 2008 adult return). Reproductive success (RS) is the number of assigned juveniles per sampled adult (or assigned parent). Note that RRS is standardized to the numerator: N_{RS} – natural origin and H_{RS} – hatchery origin. “Candidates” represents all fish sampled at the weir, while “parents” is a subset that includes only those assigned to at least one sampled juvenile; excluding adults with zero sampled offspring. Results favoring natural-origin adults and/or parents are bolded and shaded.

sampled adults		candidates (n)	offspring assigned (n)	RS	RRS		parents (n)	offspring assigned (n)	RS	RRS		
sex	origin				(N_{RS}/H_{RS})	(H_{RS}/N_{RS})				(N_{RS}/H_{RS})	(H_{RS}/N_{RS})	
2009 Fall outmigrants: pre-smolts												
M	HAT	18	10	0.56	---	2.33	4	10	2.50	---	3.00	
	NOR	21	5	0.24	0.43	---	6	5	0.83	0.33	---	
	Stray	0	---	---	---	---	0	---	---	---	---	
F	HAT	3	13	4.33	---	6.81	3	13	4.33	---	3.10	
	NOR	11	7	0.64	0.15	---	5	7	1.40	0.32	---	
	Stray	2	1	0.50	---	---	1	1	1.00	---	---	
combined	HAT	21	23	1.10	---	2.92	7	23	3.29	---	3.01	
	NOR	32	12	0.38	0.34	---	11	12	1.09	0.33	---	
	Stray	2	1	0.50	---	---	1	1	1.00	---	---	
2010 Spring outmigrants: smolts												
M	HAT	18	5	0.28	---	0.32	4	5	1.25	---	0.42	
	NOR	21	18	0.86	3.09	---	6	18	3.00	2.40	---	
	Stray	0	---	---	---	---	0	---	---	---	---	
F	HAT	3	2	0.67	---	0.27	3	2	0.67	---	0.12	
	NOR	11	27	2.45	3.68	---	5	27	5.40	8.10	---	
	Stray	2	0	0.00	---	---	1	0	0.00	---	---	
combined	HAT	21	7	0.33	---	0.24	7	7	1.00	---	0.24	
	NOR	32	45	1.41	4.22	---	11	45	4.09	4.09	---	
	Stray	2	0	0.00	---	---	1	0	0.00	---	---	

Table 2b. RRS summary for brood year 2009 (i.e. the 2009 adult return). An RRS was not calculated for strays, and no female stray adults were identified among the return in 2009. Only a single (*) female hatchery-origin adult was observed, which did not produce any sampled offspring; therefore, calculation of RRS for females was not possible for BY2009. Results favoring natural-origin adults and/or parents are bolded and shaded.

sampled adults		candidates	offspring	RRS			parents	offspring	RRS		
sex	origin	(n)	assigned (n)	RS	(N_{RS}/H_{RS})	(H_{RS}/N_{RS})	(n)	assigned (n)	RS	(N_{RS}/H_{RS})	(H_{RS}/N_{RS})
2010 Fall outmigrants: pre-smolts											
M	HAT	7	32	4.57	---	2.54	5	32	6.40	---	1.42
	NOR	10	18	1.80	0.39	---	4	18	4.50	0.70	---
	Stray	6	76	12.67	---	---	4	76	19.00	---	---
F	HAT	1*	0	0.00	---	0.00	0	0	---	---	---
	NOR	12	131	10.92	NA	---	4	131	32.75	NA	---
	Stray	0	---	---	---	---	0	---	---	---	---
combined	HAT	8	32	4.00	---	0.59	5	32	6.40	---	0.34
	NOR	22	149	6.77	1.69	---	8	149	18.63	2.91	---
	Stray	6	76	12.67	---	---	4	76	19.00	---	---
2011 Spring outmigrants: smolts											
M	HAT	7	2	0.29	---	2.86	5	2	0.40	---	1.60
	NOR	10	1	0.10	0.35	---	4	1	0.25	0.63	---
	Stray	6	5	0.83	---	---	4	5	1.25	---	---
F	HAT	1*	0	0.00	---	0.00	0	0	---	---	---
	NOR	12	9	0.75	NA	---	4	9	2.25	NA	---
	Stray	0	---	---	---	---	0	---	---	---	---
combined	HAT	8	2	0.25	---	0.55	5	2	0.40	---	0.32
	NOR	22	10	0.45	1.82	---	8	10	1.25	3.13	---
	Stray	6	5	0.83	---	---	4	5	1.25	---	---

Table 2c. RRS summary for brood year 2010 (i.e. the 2010 adult return). One adult stray male and one stray female were identified in the 2010 return; neither was identified as a parent and both were excluded from analyses. A group of sampled juveniles were identified as precocious parr (i.e. release of milt). Results favoring natural-origin adults and/or parents are bolded and shaded.

sex	sampled adults origin	candidates (n)	offspring assigned (n)	RRS			parents (n)	offspring assigned (n)	RRS		
				RS	(N _{RS} /H _{RS})	(H _{RS} /N _{RS})			RS	(N _{RS} /H _{RS})	(H _{RS} /N _{RS})
2011 Fall outmigrants: pre-smolts											
M	HAT	68	237	3.5	---	2.06	39	237	6.1	---	1.93
	NOR	13	22	1.7	0.49	---	7	22	3.1	0.52	---
F	HAT	52	276	5.3	---	0.48	33	276	8.4	---	0.63
	NOR	6	66	11.0	2.07	---	5	66	13.2	1.58	---
combined	HAT	120	513	4.3	---	0.92	72	513	7.1	---	0.97
	NOR	19	88	4.6	1.08	---	12	88	7.3	1.03	---
2012 Spring outmigrants: smolts											
M	HAT	68	100	1.5	---	0.87	39	100	2.6	---	0.82
	NOR	13	22	1.7	1.15	---	7	22	3.1	1.23	---
F	HAT	52	208	4.0	---	1.41	33	208	6.3	---	1.85
	NOR	6	17	2.8	0.71	---	5	17	3.4	0.54	---
combined	HAT	120	308	2.6	---	1.25	72	308	4.3	---	1.32
	NOR	19	39	2.1	0.80	---	12	39	3.3	0.76	---
2012 Fall outmigrants: precocial											
M	HAT	68	31	0.5	---	1.48	39	31	0.8	---	1.39
	NOR	13	4	0.3	0.67	---	7	4	0.6	0.72	---
F	HAT	52	51	1.0	---	0.35	33	51	1.5	---	0.45
	NOR	6	17	2.8	2.89	---	5	17	3.4	2.20	---
combined	HAT	120	82	0.7	---	0.62	72	82	1.1	---	0.65
	NOR	19	21	1.1	1.62	---	12	21	1.8	1.54	---

Table 3. Geo-means for RRS at pre-smolt and smolt life stages. Summed results (BY2008 through BY2010) correspond with Table 2a-c that summarize reproductive success for each brood year individually. Because there were no results for hatchery females in 2009 the geo-means for females and “combined” represent only BY2008 and BY2010. Note however that RS for natural-origin females [and combined RRS (N_{RS}/H_{RS})] was high for BY2009 (see Table 2b). Results favoring natural-origin adults and/or parents are bolded and shaded.

RRS	<u>Adult Male</u>		<u>Adult Female</u>		<u>combined</u>	
	(N_{RS}/H_{RS})	(H_{RS}/N_{RS})	(N_{RS}/H_{RS})	(H_{RS}/N_{RS})	(N_{RS}/H_{RS})	(H_{RS}/N_{RS})
<u>total sample</u>						
presmolts	0.47	2.13	1.71	0.58	1.02	0.98
smolts	1.32	0.84	0.97	1.03	0.99	1.01
<u>parents only</u>						
presmolts	0.53	1.91	1.41	0.71	0.97	1.03
smolts	1.33	0.79	0.91	1.10	0.95	1.06

Table 4. Assigned parent pairs identified by type of cross (H – hatchery, N – natural, ST – stray). The number (n) of assigned pairs is the sum among brood years (BY2008-BY2010). The number and proportion (%n) of offspring by age category is the sum among brood year. For H –X- N crosses 77% of pre-smolt assignments (*) occurred when the male parent was hatchery origin.

<u>observed crosses</u>		<u>offspring (n)</u>			
type	(n)	pre-smolt	smolt	precocial	total
H -X- H	58	(n) 170 % 0.68	67 0.27	12 0.05	249 ---
H -X- N	27	(n) *64 % 0.60	28 0.26	15 0.14	107 ---
N -X- N	14	(n) 12 % 0.36	20 0.61	1 0.03	33 ---
N -X- ST	5	(n) 40 % 0.98	1 0.02	0 0	41 ---

Table 5. Adult-to-adult parentage summary: MM - # mismatched loci, (*) LOD score confidence at 99% threshold. Red text indicates error likely associated with kinship. Assignments are presumed to represent natural productivity (naturally spawning parent whether hatchery- or natural-origin). Therefore offspring identified as hatchery-origin (gray fill) represent ID error.

Offspring ID		1st candidate					2nd candidate					Trio	
ID	origin	ID	origin	sex	MM	LOD	ID	origin	sex	MM	LOD	MM	LOD
12-0006	N	08-0007	N	F	0	21.5*	08-0014	N	M	0	12.5*	0	49.0
12-0013	N	08-0014	N	M	0	26.2*	08-0055	N	F	0	18.0*	0	50.4
12-0001	N	08-0192	H	M	0	9.7*	08-0750	H	F	0	29.6*	0	52.4
12-0072	N	08-0192	H	M	1	7.6*	08-0750	H	F	0	28.2*	1	49.7
12-0105	N	08-0701	H	M	0	11.2*	08-0750	H	F	0	23.8*	0	45.9
12-0006	N	08-0007	N	F	0	21.5*							
12-0007	N	08-0011	N	F	0	30.6*							
12-0095	N	08-0011	N	F	0	26.8*							
12-0174	N	08-0011	N	F	0	32.3*							
12-0013	N	08-0014	N	M	0	26.2*							
12-0100	N	08-0021	N	M	0	23.4*							
12-0102	N	08-0021	N	M	0	22.0*							
12-0171	N	08-0021	N	M	0	18.5*							
12-0003	N	08-0073	N	F	0	18.3*							
12-0021	N	08-0073	N	F	0	17.1*							
12-0029	N	08-0073	N	F	0	19.0*							
12-0085	N	08-0073	N	F	0	18.2*							
12-0024	H	08-0519	N	M	0	22.2*							
12-0015	H	08-0601	N	M	0	18.0*							
12-0076	H	08-0601	N	M	0	16.1*							
12-0016	N	08-0603	N	F	0	17.7*							
12-0042	H	08-0603	N	F	0	16.6*							
12-0107	H	08-0603	N	F	0	16.9*							
12-0112	N	08-0603	N	F	0	18.5*							
12-0001	N	08-0750	H	F	0	29.6*							
12-0072	N	08-0750	H	F	0	28.2*							
12-0169	N	08-0750	H	F	0	20.8*							
12-0071	N	11-0048	H	F	0	17.7*	(Not Possible)						
12-0075	N	11-0048	H	F	0	21.7*	(Not Possible)						
12-0010	H	11-0252	H	M	0	22.5*	(Not Possible)						

Figure 1. Proportion of observed female parents (BY2008-BY2010) that produced a corresponding number of assigned juvenile offspring; precocials are BY2010 only.

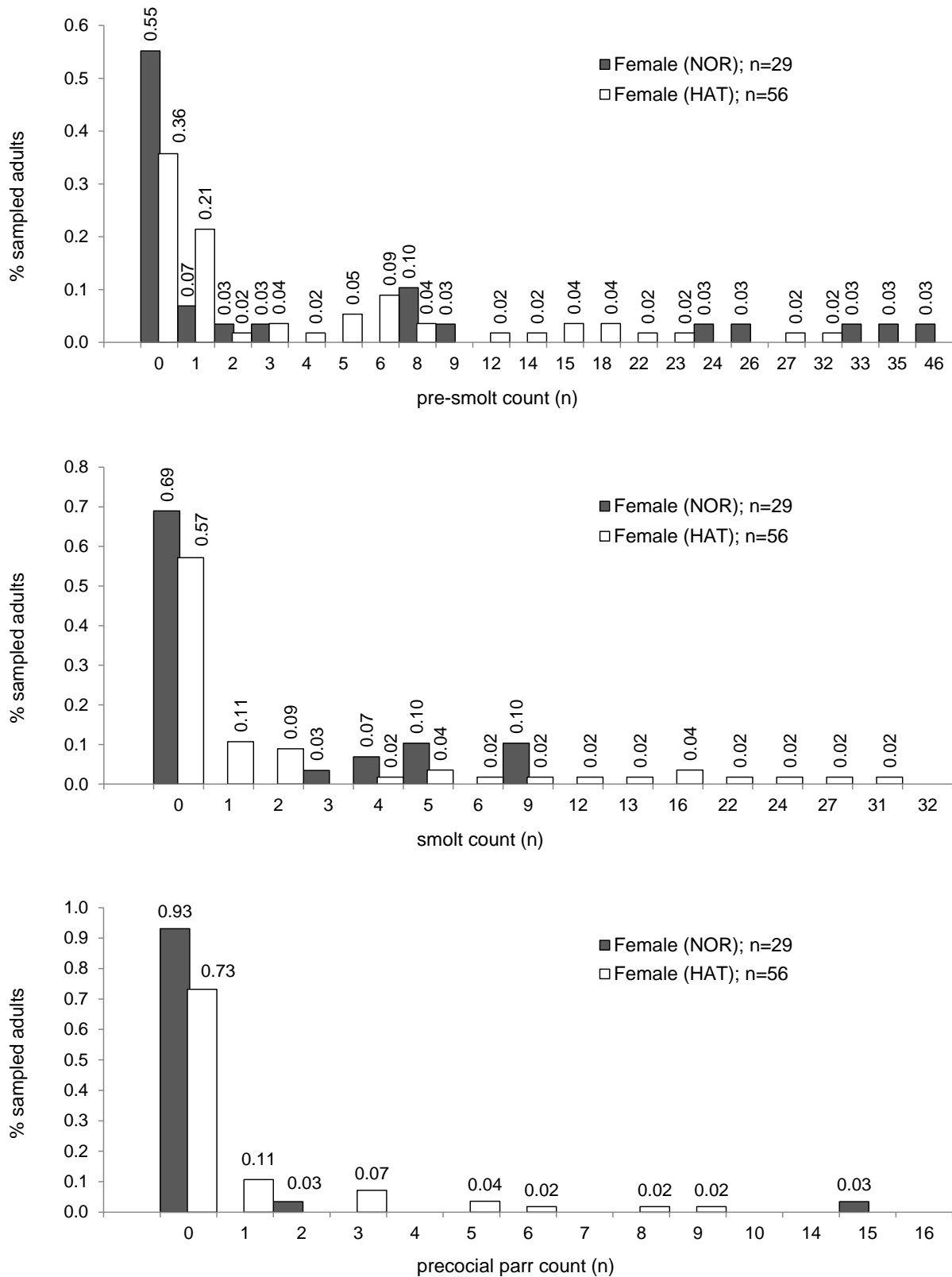


Figure 1 continued. BY08-BY10 results for male parents.

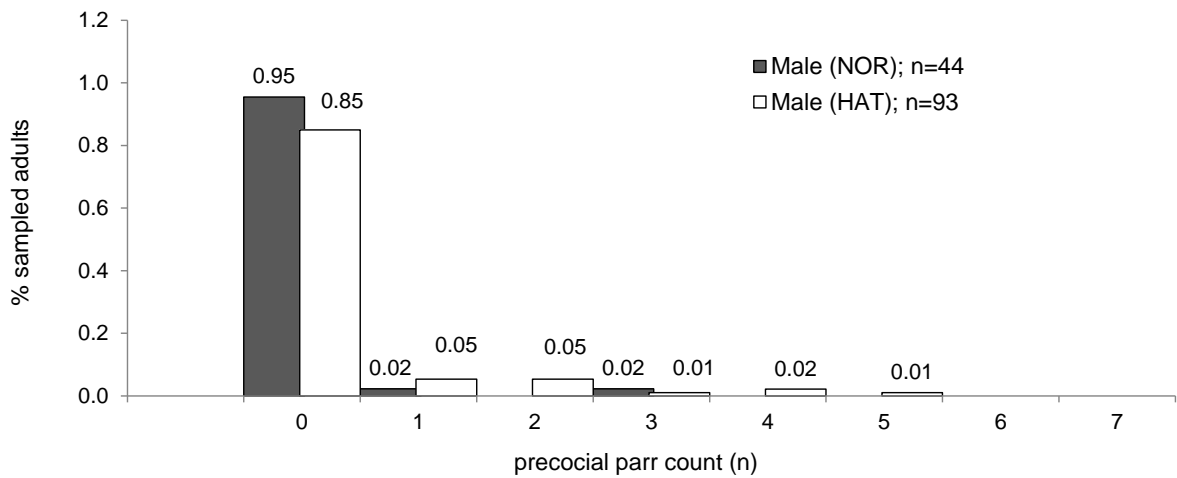
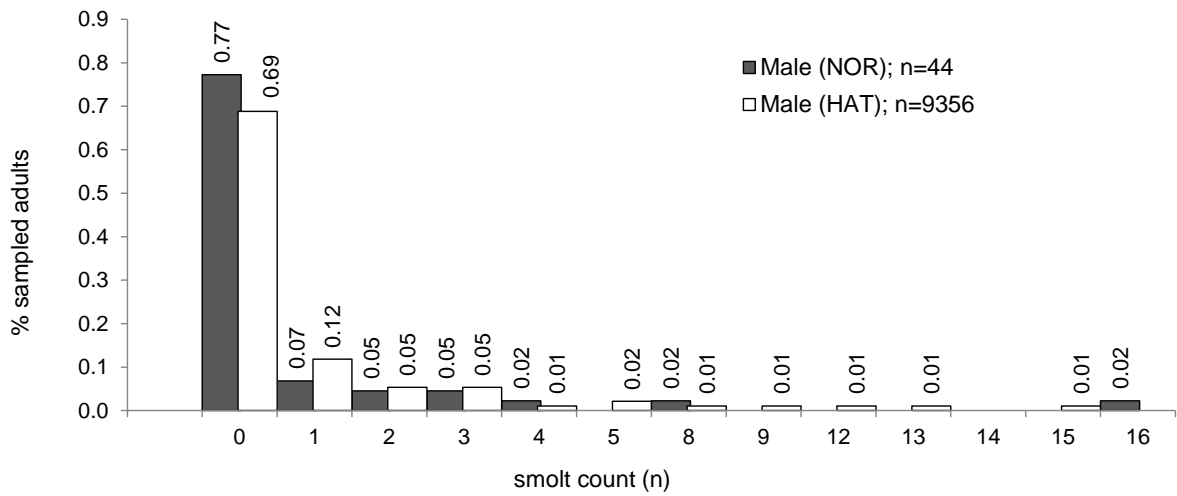
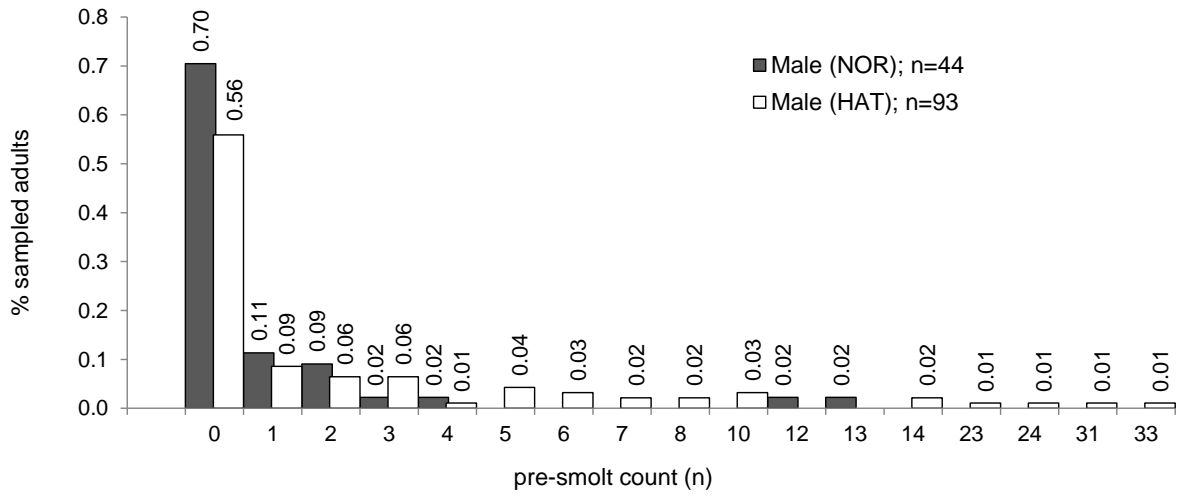


Figure 2. Size-at-age based on parentage assignments. Fall 2012 outmigrants are putative BY2011 pre-smolts (or BY2010 precocial parr), while Spring 2012 outmigrants are putative BY2010 smolts. Age categories were indeed corroborated through genetic parentage analysis.

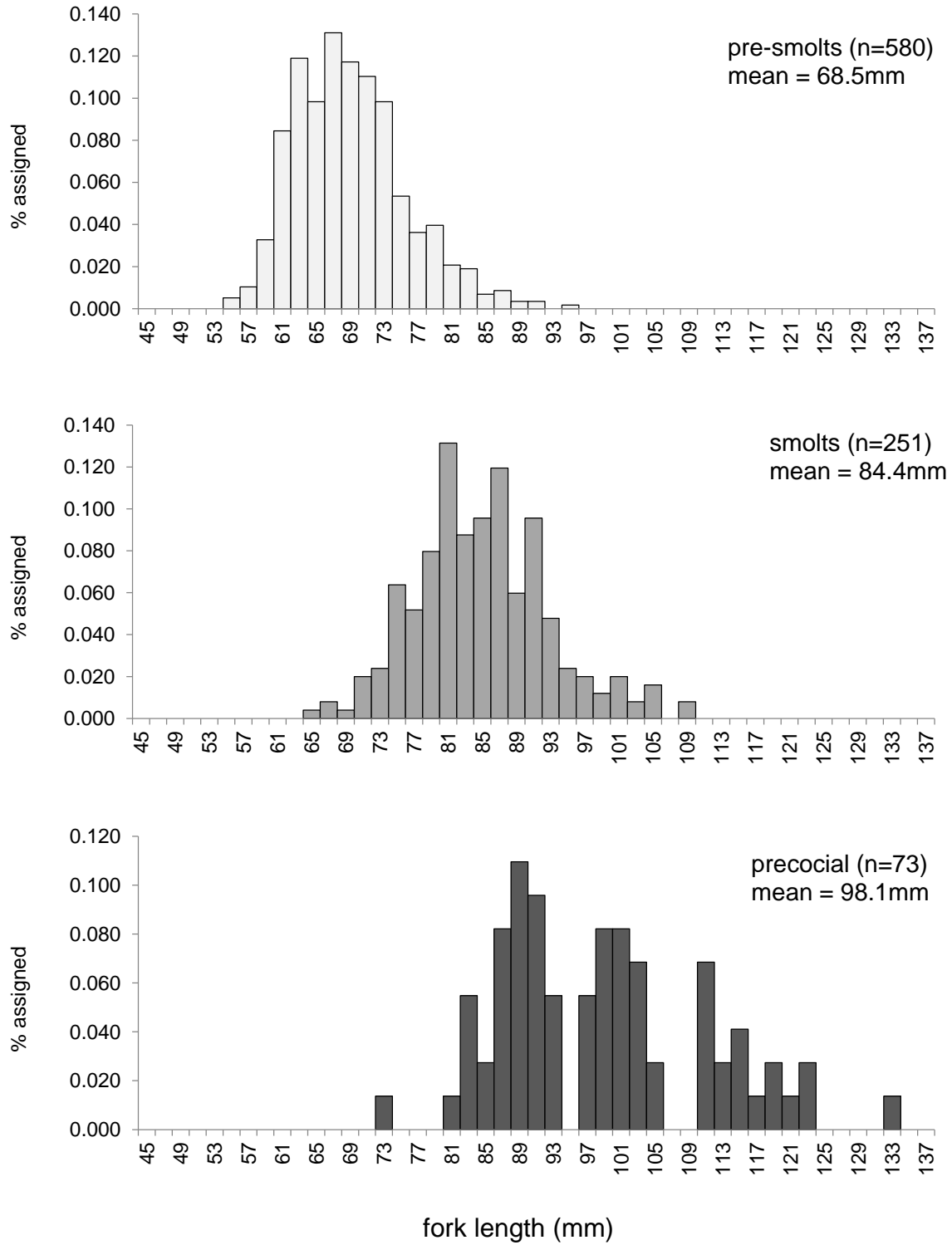
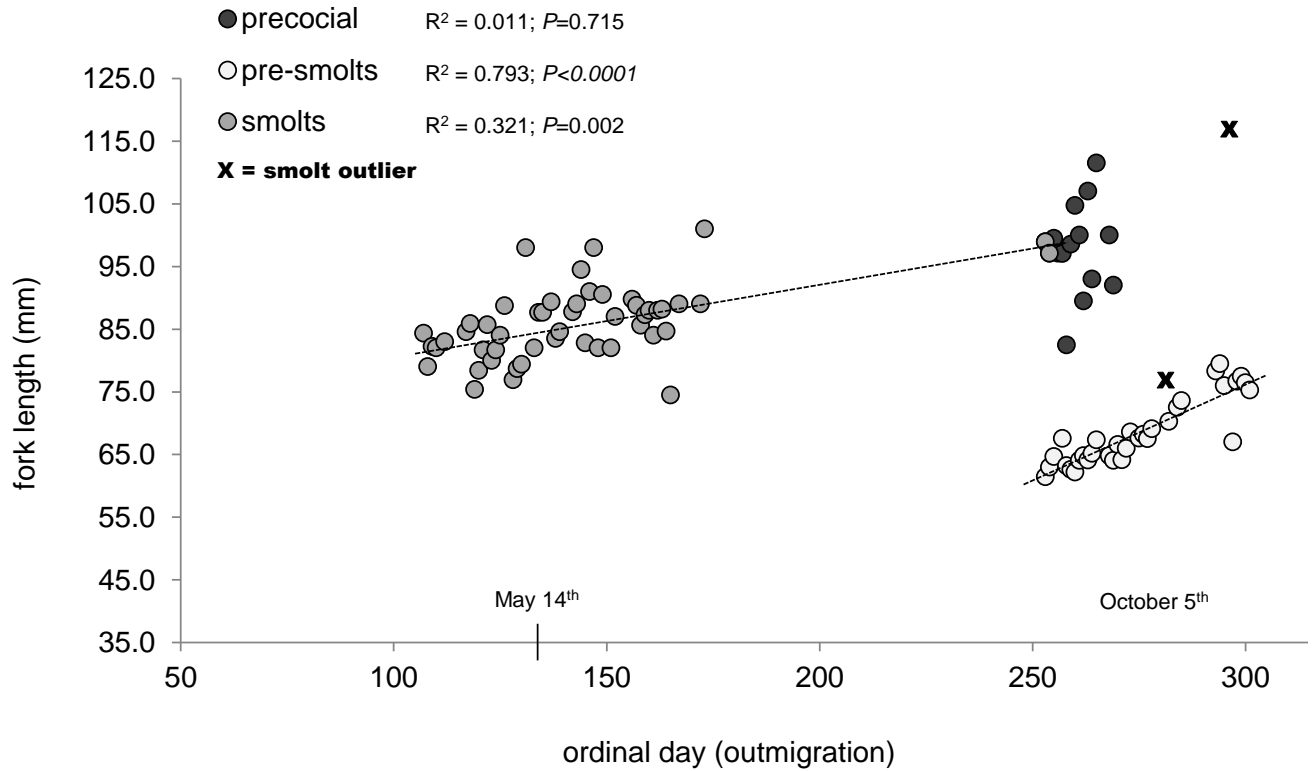


Figure 3. Size-at-age based on parentage assignments. Fall 2012 outmigrants are putative BY2011 pre-smolts (or BY2010 precocial parr), while spring 2012 outmigrants are putative BY2010 smolts. Growth trajectories show increasing size by age class across respective outmigration periods.



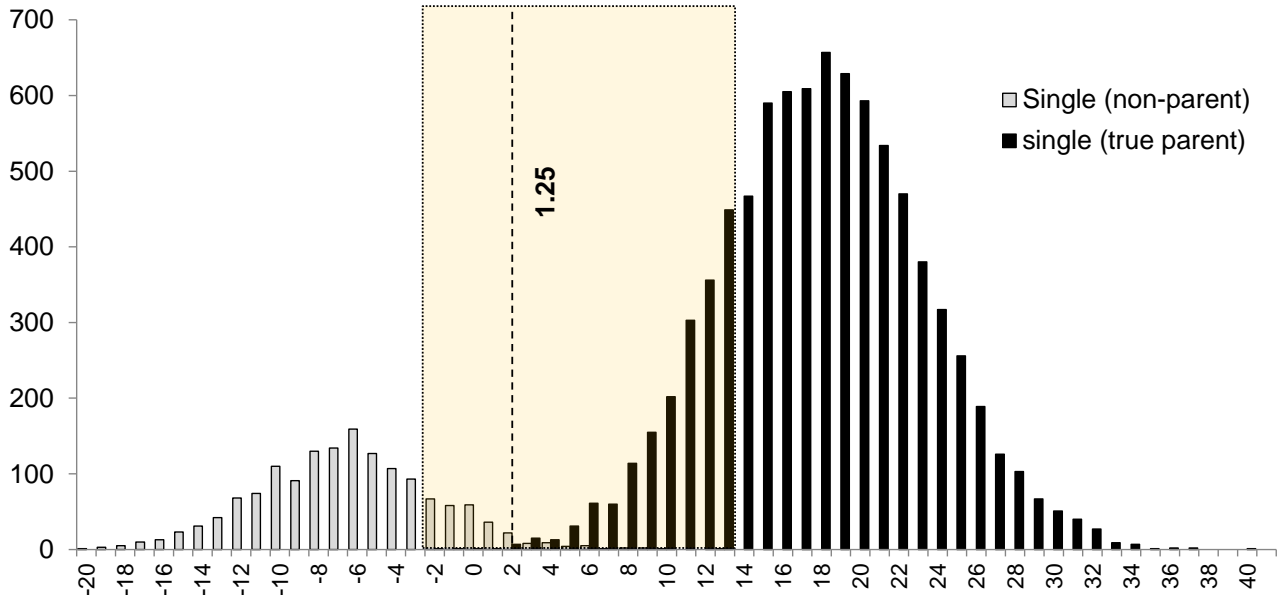
Appendix 1. Parentage results using the parentage based tagging (PBT) baseline for Chinook salmon throughout the Snake River. Bolded ID numbers are jacks (e.g. 2011 smolt releases from BY09 McCall stock). Note that a significant number of fish that were field identified as natural origin (N) are the progeny of hatchery broodstocks, primarily from NPTH. In subsequently parentage analyses where 2012 collections are to be treated as candidate parents, the origin ID of these individuals will need to be corrected to avoid an upward bias on natural-origin reproductive success.

<u>PBT assigned parents</u>			<u>assignment statistics</u>			<u>Offspring (Newsome weir adults)</u>					
<u>father</u>	<u>mother</u>	<u>PBT hatchery /site</u>	<u>FDR</u>	<u>P-value</u>	<u>LOD</u>	<u>ID</u>	<u>Date</u>	<u>Year</u>	<u>Gender</u>	<u>Length</u>	<u>Origin</u>
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.7	11-0048	07/19/11	2011	M	470	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.5	11-0250	07/14/11	2011	M	420	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	22.7	11-0265	07/16/11	2011	M	410	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00400	19.6	11-0281	07/17/11	2011	M	380	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.1	11-0285	07/18/11	2011	M	380	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.2	11-0343	08/01/11	2011	M	440	HAT
2009	2009	<i>Clearwater Fish Hatchery</i>	0.75054	0.02300	18.8	12-0197	09/04/12	2012	M	495	HAT
2009	2009	<i>Clearwater Fish Hatchery</i>	0.00000	0.00000	21.6	12-0196	09/03/12	2012	M	485	N
2009	2009	<i>McCall Fish Hatchery</i>	0.00005	0.01200	18.1	12-0198	09/06/12	2012	M	500	HAT
2009	2009	<i>McCall Fish Hatchery</i>	0.00001	0.00100	20.0	12-0199	09/06/12	2012	M	512	HAT
2009	2009	<i>McCall Fish Hatchery</i>	0.00002	0.00300	20.0	12-0184	07/20/12	2012	F	530	HAT
2009	2009	<i>McCall Fish Hatchery</i>	0.00003	0.00600	17.2	12-0173	07/14/12	2012	M	532	HAT
2009	2009	<i>McCall Fish Hatchery</i>	0.00002	0.00200	19.7	12-0190	08/05/12	2012	M	545	HAT
2009	2009	<i>McCall Fish Hatchery</i>	0.00000	0.00000	19.3	12-0175	07/15/12	2012	M	550	HAT
2009	2009	<i>McCall Fish Hatchery</i>	0.00008	0.02300	18.8	12-0200	09/08/12	2012	M	580	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00200	20.1	12-0036	06/24/12	2012	F	590	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.5	12-0098	07/05/12	2012	M	620	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.6	12-0183	07/19/12	2012	F	620	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00400	19.1	12-0083	07/02/12	2012	F	640	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.4	12-0055	06/29/12	2012	F	646	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.2	12-0084	07/02/12	2012	F	666	HAT

2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	19.8	12-0017	06/23/12	2012	F	667	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.3	12-0032	06/24/12	2012	F	675	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	21.4	12-0109	07/09/12	2012	F	675	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.4	12-0037	06/24/12	2012	F	680	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00300	20.2	12-0040	06/25/12	2012	F	680	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.8	12-0077	07/02/12	2012	F	681	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.5	12-0048	06/26/12	2012	M	685	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.4	12-0094	07/04/12	2012	F	685	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00001	0.01400	18.3	12-0039	06/25/12	2012	F	690	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00004	0.04800	16.3	12-0056	06/29/12	2012	F	690	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.2	12-0089	07/03/12	2012	F	699	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	21.6	12-0057	06/29/12	2012	M	700	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00001	0.00600	19.2	12-0249	09/04/12	2012	F	705	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.3	12-0081	07/02/12	2012	F	709	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	19.7	12-0062	06/29/12	2012	F	710	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.7	12-0179	07/17/12	2012	M	711	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	22.7	12-0050	06/26/12	2012	F	713	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	20.1	12-0012	06/22/12	2012	F	720	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.3	12-0065	06/30/12	2012	M	720	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00300	19.3	12-0033	06/24/12	2012	F	723	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00200	20.5	12-0068	06/30/12	2012	F	727	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	21.0	12-0067	06/30/12	2012	F	729	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.2	12-0045	06/25/12	2012	M	733	HAT
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	22.0	12-0087	07/03/12	2012	M	772	HAT
2008	2008	<i>Powell Satellite Fish Hatchery</i>	0.02009	0.00200	19.1	12-0080	07/02/12	2012	F	767	HAT
2008	2008	<i>Clearwater Fish Hatchery</i>	0.00000	0.00000	20.3	12-0201	09/08/12	2012	M	640	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00100	20.4	12-0099	07/05/12	2012	F	645	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00400	20.4	12-0064	06/30/12	2012	F	662	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.1	12-0066	06/30/12	2012	M	663	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	22.1	12-0044	06/25/12	2012	F	674	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.2	12-0063	06/30/12	2012	F	684	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00001	0.01500	19.1	12-0035	06/24/12	2012	F	688	N

2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.3	12-0027	06/24/12	2012	M	690	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.0	12-0046	06/25/12	2012	F	690	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00001	0.01500	18.2	12-0091	07/03/12	2012	F	690	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.2	12-0071	07/01/12	2012	M	706	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.0	12-0060	06/29/12	2012	F	707	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	22.2	12-0073	07/01/12	2012	M	727	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.6	12-0075	07/01/12	2012	F	734	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	22.3	12-0009	06/19/12	2012	F	735	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	19.6	12-0020	06/23/12	2012	M	735	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.2	12-0092	07/03/12	2012	F	735	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00200	19.9	12-0192	08/30/12	2012	M	740	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00001	0.00800	18.9	12-0088	07/03/12	2012	M	765	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	20.8	12-0031	06/24/12	2012	F	775	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00200	19.4	12-0038	06/25/12	2012	F	780	N
2008	2008	Nez Perce Tribal Fish Hatchery	0.00000	0.00000	21.3	12-0019	06/23/12	2012	M	980	N

Supplemental 1a. Parentage simulation results identifying the log odds ratio (LOD) distributions between single parent mis-assignment (non-parent) and single parent assignment (true parent). The shaded area defines the overlap in the tails of the distributions. The bolded value and dashed vertical line identify the strict confidence LOD (99%).



Supplemental 1b. Parentage simulation results identifying the log odds ratio (LOD) distributions between parent pair mis-assignment (non-parent pair) and parent pair assignment (true parent pair). The shaded area defines the overlap in the tails of the distributions. The bolded value and dashed vertical line identify the strict confidence LOD (99%).

