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Yakima/Klickitat Fisheries Project Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

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This report covers one of many topics under the Yakima/Klickitat Fisheries Project's Monitoring and Evaluation Program (YKFPME). The YKFPME is funded under two BPA contracts, one for the Yakama Nation and the other for the Washington Department of Fish and Wildlife (contract number 00053279, Project Number 1995-063-25). A comprehensive summary report for all of the monitoring and evaluation topics will be submitted after all of the topical reports are completed. This approach to reporting enhances the ability of people to get the information they want, enhances timely reporting of results, and provides a condensed synthesis of the whole YKFPME. The current report was completed by the Washington Department of Fish and Wildlife.

Executive Summary

Chapter 1: A population-of-origin assignment procedure was used to estimate the percentages of unknown-origin smolts from each of five stock groups outmigrating past Chandler Trap (Yakima River) from January – July 2011. Mixture analysis was conducted on a proportional subsample of 1,045 smolts collected during the outmigration at Chandler Trap. Assignment of each individual to a population-of-origin was determined if the posterior probability of the assignment was greater than 90.0%. The largest percentage of outmigrating smolts in the January/February, March, April, and May time strata was from the upper Yakima River stock while the June – July time stratum was dominated by the fall stocks with 84.2% of the total assignments. Comparison of morphological assessment and genetic assignment as a spring or fall Chinook smolt conducted for all time strata indicated agreement for 830/843 (98.5%) of the smolts.

Chapter 2: We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of Chinook salmon spawners from the hatchery-control line (two generations of hatchery influence) and the supplementation hatchery line (SH – one generation of hatchery influence) in the Cle Elum experimental spawning channel for the 2010 brood year. The assignments were based on offspring genotypes at 14 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999999. Two thousand five hundred and fifty-nine of 2,920 fry from the 2010 brood that were genotyped at eight or more loci were assigned to a parental pair with 95% confidence. The number of progeny attributed to individual potential parents was quite variable, ranging from 0 to 333 for all males and from 0 to 165 for females. The sum of progeny attributed to the hatchery-control line males and females was 1,180, while the sum of progeny attributed to supplementation hatchery line males and females was 1,419.

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Chapter 1

DNA-Based Population-of-Origin Assignments of Chinook Salmon Smolts Outmigrating Past Chandler Trap at Prosser Dam (Yakima River) in 2011

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Abstract

A population-of-origin assignment procedure was used to estimate the percentages of unknown-origin smolts from each of five stock groups outmigrating past Chandler Trap (Yakima River) from January – July 2011. Mixture analysis was conducted on a proportional subsample of 1,045 smolts collected during the outmigration at Chandler Trap. Assignment of each individual to a population-of-origin was determined if the posterior probability of the assignment was greater than 90.0%. The largest percentage of outmigrating smolts in the January/February, March, April, and May time strata was from the upper Yakima River stock while the June – July time stratum was dominated by the fall stocks with 84.2% of the total assignments. Comparison of morphological assessment and genetic assignment as a spring or fall Chinook smolt conducted for all time strata indicated agreement for 830/843 (98.5%) of the smolts.

Introduction

Production and survival of the Yakima River basin spring Chinook stocks (American River, Naches River, and upper Yakima River) are monitored, as part of the Yakima/Klickitat Fishery Project supplementation evaluation program. However, in the lower Yakima River, where the best facilities to collect samples exist, the three spring Chinook stocks are mixed with one another and with the Marion Drain and Yakima River fall Chinook stocks, during downstream juvenile migration. Thus, methodologies for discriminating stocks in an admixture are vital for development of stock-specific estimates. Domestication monitoring plans require discrimination of the three spring Chinook salmon stocks in the basin, and a complete analysis of migration timing and stock abundance for all Chinook requires discrimination of the two fall stocks as well. Accurate assignments of Chinook smolts captured at the Chandler fish passage facility to population-of-origin will allow researchers and managers to estimate production by the three spring Chinook stocks, assess smolt-to-smolt survival of the three spring Chinook stocks, and could be utilized to evaluate stock-specific environmental condition factors.

The methodology used in this study to estimate the population-of-origin for individual fish in a mixture followed a Bayesian approach by Rannala and Mountain (1997). This approach assumes linkage equilibrium among loci and uses the multilocus genotype of an individual to compute the probability of that genotype belonging to a population in the baseline. Others have used the methodology developed by Rannala and Mountain (1997) to provide robust population-of-origin assignments of unknown individuals (Hauser et al. 2006, Taylor and Costello 2006, and Waples and Gaggiotti 2006).

Calculation of population-of-origin for Chinook smolts trapped at Chandler trap throughout the entire outmigration (January through July) was hindered in the first few years of analysis for several reasons: non-representative temporal sampling of the downstream migration, past omission of the Marion Drain fall and lower Yakima River mainstem fall Chinook stocks from the DNA baseline, and by maintenance and other shutdowns of trap operations in December and January in many years. In the analyses of samples from 2004 - 2010, attempts were made to eliminate the problems present in previous analyses. A new sampling design was initiated to provide a proportional sample of smolts outmigrating past Chandler trap and a larger number of smolts were analyzed. Repeated multi-year samples of all five baseline stocks were used to characterize the potential sources of smolts in the Yakima River basin.

This report presents the population-of-origin assignments for outmigrating smolts collected at the Chandler trap during 2011.

Materials and Methods

Collections

There were no collections added to the Yakima River baseline this year. Since 1989, sampling crews from the Yakama Nation and WDFW have collected adult spawning ground tissue samples to be included in the baseline. The tissue samples consisted of dry-mounted scales or fin tissue preserved in 100% ethanol from five baseline stocks collected across multiple years

(American River spring, Naches River spring, upper Yakima River spring, Marion Drain fall, and lower Yakima River fall; Table 1 and Figure 1).

An estimated total of 827,024 smolts passed the lower Yakima River at Chandler from January 14 – July 20, 2011. This estimate was based on expansion of the total number of smolts counted at the Chandler trap (32,566) to account for trap efficiency, etc. Unknown-origin smolts were collected at Prosser Dam (Chandler Trap) following a sampling design that would identify a proportional number of smolt samples that represents the entire smolt outmigration. The following four time strata (January – February, April, May, and June – July) were used for analysis. Samples were collected from January 5 – July 30, 2011. These samples were genetically analyzed to get reliable estimates of population proportions. Each day, the total number of smolts at the trap was visually estimated before any processing occurred. If that number was below a predetermined threshold then a "standard" day's sample was taken (e.g. 10 fish). If the number of smolts was above the threshold then a "peak" day's sample was taken (e.g. 30 fish). The threshold for "standard" and "peak" days and the numbers of samples to be taken on each day varied for each of the time strata. These values were determined by analyzing the number of "peak" and "standard" days counted during four years of smolt outmigration monitoring. Based on this sampling design, 2,551 Chinook smolt samples were collected for genetic analysis.

The total estimated numbers of smolts passing the Chandler Trap each day were plotted with the total number of genetic samples that had been collected. A process was then employed to proportionalize the available genetic samples with the daily counts to provide a representative number of smolts that were outmigrating from January – July. A total of 1,045 smolts were identified for analysis.

DNA Extraction Methods

Genomic DNA was extracted by digesting a small piece of fin tissue (all smolt and some adult baseline collections) or scales (most adult baseline collections) using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

PCR Methods

The polymerase chain reaction mixture contained the following for a 10 µL reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units Go*Taq* Flexi DNA polymerase (Promega). Amplification was performed using MJ Research PTC-200 and Applied Biosystems 9700 thermocyclers. The thermal profile was as follows: an initial denaturation step of 2 minutes at 94^{o}C; 40 cycles of 15 seconds at 94^{o}C, 30 seconds at 50-60^{o}C, and 1 minute at 72^{o}C; plus a final extension step at 72^{o}C for 10 minutes, followed by a final indefinite holding step at 10^{o}C.

Eleven microsatellite DNA loci (Table 2) were amplified via the polymerase chain reaction (PCR) using fluorescently labeled primers (obtained from Applied Biosystems or Integrated DNA Technologies). Loci were combined in multiplexes to increase efficiency and decrease costs.

Data were collected using an AB-3730 Genetic Analyzer. Applied Biosystems GENEMAPPER v.3.7 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et. al., 2007).

Population-of-origin Analysis
Population-of-origin assignments for the analysis of unknown-origin smolts were accomplished with GMA (Kalinowski 2003) using a Bayesian Method described by Rannala and Mountain (1997). GMA estimates the probability of an individual coming from a baseline population based on the population's estimated contribution to the mixture as a prior (Kalinowski 2003). All assignments with a posterior probability greater than or equal to 90% were accepted. Because GMA uses the estimated contribution as a prior the analysis of the known-origin samples was conducted using approximately equal numbers of samples.

Comparison of Morphological ID and Genetic Assignment
Smolts were categorized as spring or fall Chinook when they were intercepted at the Chandler Trap based on morphological characteristics. Three morphological features (length, size of the eye, and snout shape) were used to identify smolts as spring or fall (Mark Johnston, Yakama Nation; pers. comm.).

Results

Collections
A total of 1,045 unknown Chinook smolts were selected and analyzed from those collected at Chandler Trap. Smolt samples that were missing data for six or more loci (N = 19) were dropped from statistical analyses.

Population-of-origin Analysis
The mixture composition estimates for the entire 2011 smolt outmigration indicated that the largest overall percentage of spring smolts was from the upper Yakima River followed by the Naches River and American River in the first four strata. During the migration from January – May, the proportion of the upper Yakima River stocks was between 72.5 and 80.3% while the American River and Naches River spring stocks was between 0.0 and 24.0%. The proportion of the two fall stocks was between 0.0 – 2.0% for the first four time strata and 84.2% in the June - July time stratum (Table 3).

Comparison of Morphological ID and Genetic Assignment
A comparison of the morphological assessment to genetic assignment was conducted for all five time strata. A total of 179 smolts in January/February, 53 smolts in March, 445 smolts in April, 105 smolts in May, and 61 in the June/July time strata were scored, and therefore included in the analysis. Results for the time strata were as follows: January/February time stratum – all 179 smolts were assigned identically using morphological and genetic methods (179 spring); March stratum – all 53 smolts were assigned identically using morphological and genetic methods (53 spring); April time stratum – 445 smolts were assigned identically using morphological and

genetic methods (445 spring); May time stratum – 104 out of 105 smolts were assigned identically using morphological and genetic methods (103 spring – 1 fall), the discrepancy was a assigned as a fall by genetic analysis and spring by morphological identification; June/July time stratum – 49 out of 61 smolts were assigned identically using morphological and genetic methods (1 spring and 48 fall), one discrepancy was a smolt that was assigned as fall by the genetic analyses while morphological identification was spring, the remaining 11 discrepancies were identified as a fall by the genetic analysis and spring with morphological identification.

Discussion

Collection of smolts at the Chandler Trap in 2011 utilized a sampling design intended to yield a sample that was proportional to the number of smolts passing the Chandler Trap. Sampling a proportional number of smolts was important to determine an accurate percentage of smolts from each stock that were outmigrating from the basin. Developing the sampling strategy for identifying a "standard" versus "peak" day of smolts that were in the trap and applying a sampling goal for those days allowed for a proportional sample. Subsampling the smolts collected for genetic analysis provided a best fit to the actual passage of smolts for a given day.

Monitoring the relative abundances of Chinook smolts in the Yakima River from the three different populations of spring Chinook (upper Yakima River, American River, and Naches River) and the two populations of fall Chinook (Marion Drain and lower Yakima River) requires the ability to estimate population composition of smolts outmigrating past Chandler trap. Because all five Chinook populations are intermingled when they pass Chandler trap, and the vast majority are unmarked and untagged, the only way to determine population-of-origin is by genetic analysis. This method requires that sufficient genetic differences exist among these populations in the Yakima River basin.

A baseline of 19 individual collections from the five populations in the Yakima River basin was used for the population-of-origin assignments of the outmigrating smolts. The baseline collections as a whole had higher genotyping failure compared to the Chandler smolt samples. Scales were taken from carcasses on spawning grounds for most baseline collections; therefore, DNA quality was presumably poorer than the Chandler smolt collection where tissue was collected from live fish. The upper Yakima River tissue collections were also taken from live fish at the hatchery and, therefore, genotyping success was higher for this collection than the other baseline collections.

Assessment of spring or fall smolts by morphological and genetic analysis revealed good agreement between the two methods with thirteen individuals being identified differently. Identification as a spring or fall smolt was the same for 830 smolts collected during the January – February, March, April, May, and June – July time strata.

The majority of the assignments between January and May were from the three spring stocks. The upper Yakima River spring stock accounted for the highest average percentage (75.9%) of smolts present in that period. Rank in abundance of the three spring stocks was the same in the three time strata (January-February, March, April, and May) with upper Yakima River spring

stock having the most. The June-July time stratum was predominately composed of the fall Chinook stocks, accounting for over 84.0% of the total number of smolts.

Assessment of DNA Mixture Assignments from 2000 – 2010

Mixed stock analysis has been conducted on Chandler smolts since 2000 (Young 2004, Kassler et al. 2005, Kassler 2006, Kassler and VonBargen 2007, 2008, 2009 and 2010, Kassler and Peterson 2011); however the sampling design for samples collected in 2000 – 2003 was not proportionalized during the run. The yearly assignments are therefore not comparable from those years. Beginning in 2004, staff at the Chandler trap utilized a sampling protocol to provide a number of smolts that was relative to the percentage of smolts passing that day. Samples were then subsampled at WDFW to provide a proportional number of samples that would represent the overall passage to be analyzed.

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Table 1. Nineteen Chinook salmon collections assembled into a baseline and used for the analysis of the known-origin and unknown-origin smolts. "*" the 05LU collection from Marion Drain was not used in the baseline, but is listed here as a collection from Marion Drain. The percentage of single locus genotypes missing are shown for each collection.

Baseline Collections	Collection Code	# Processed	# Analyzed	% Single Locus Genotypes Missing
American River - spring	89AG	80	77	10.4%
	91DQ	102	87	9.8%
	93DO	18	17	3.2%
	03EH	100	70	6.6%
		300	251	8.6%
Naches River - spring	89AC	76	74	11.4%
	89AI	26	22	7.0%
	93DQ	50	45	6.3%
	93DR	32	25	7.3%
little Naches River - spring	04BI	42	41	2.2%
	04EM	56	45	9.9%
		282	252	7.9%
upper Yakima River - spring	92DN	24	23	5.9%
	97DA	123	115	3.9%
	03GO	99	99	1.4%
		246	237	3.0%
Marion Drain - fall	89BX	100	92	8.3%
	92FQ	92	92	5.4%
	93DY	8	8	8.0%
	05LU*	65	47	15.3%
		265	239	8.6%
lower Yakima River - fall	90DF	109	104	12.6%
	93DW	82	80	9.8%
	98FB	61	50	8.7%
		252	234	10.8%
Chandler Trap Smolts - 2011	11AO	1,045	1,026	1.0%

Table 2. Microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used in the analysis of Chinook from five stocks in the Yakima River Basin. Also included are the percent missing genotypes for both baseline and smolts collections and heterozygosity (observed (H_o) and expected (H_e)) for each locus.

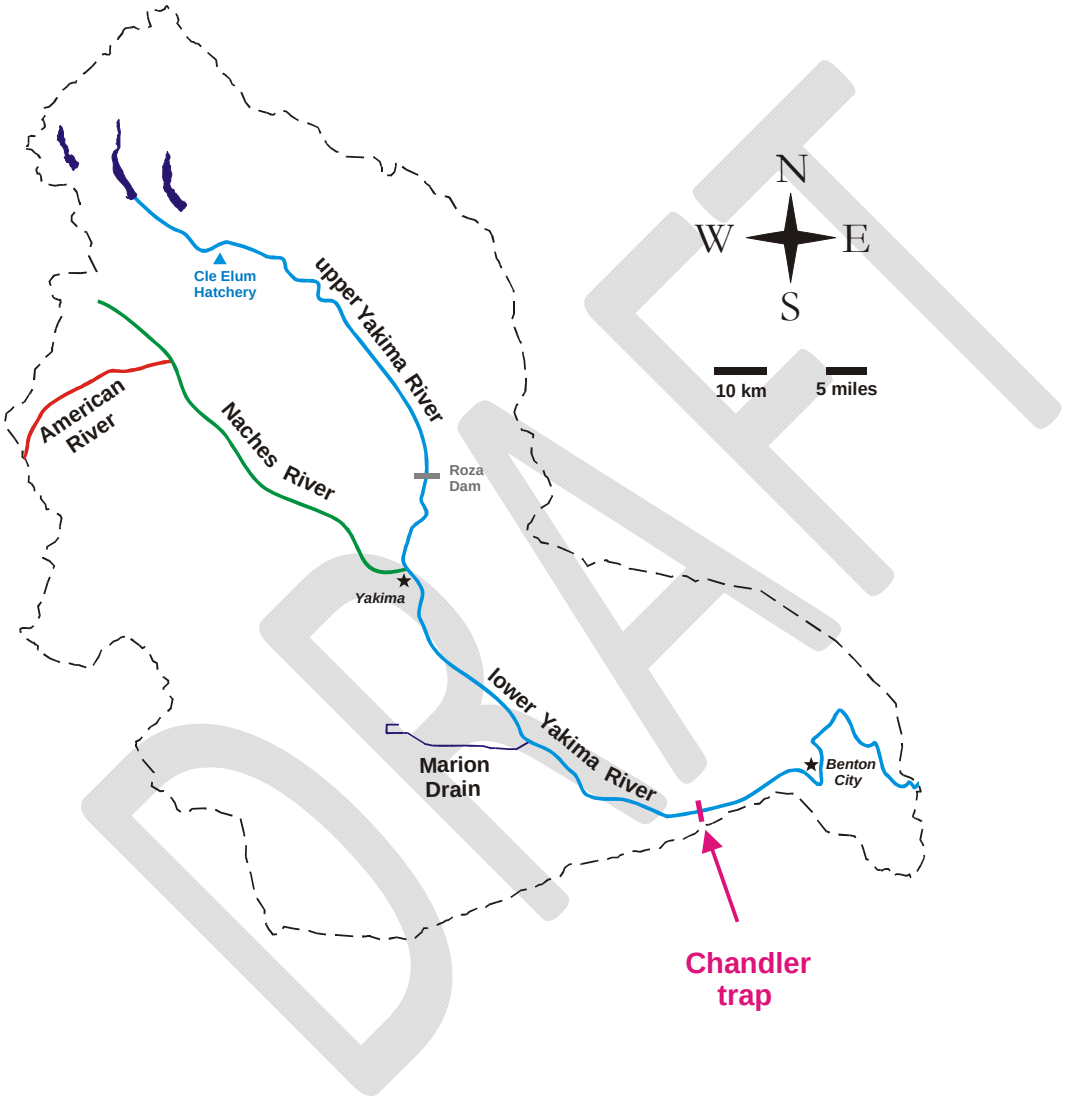
							Heterozygosity	
Multiplex	Locus	Annealing temp ^{o}C	# Alleles/ Locus	Allele Size Range (bp)	% missing genotypes baseline N = 1,213	% missing genotypes smolts N = 933	H_o	H_e
Ots-M	*Oki-100* [a]	50	41	164 - 365	11.4	5.7	0.913	0.940
	Ots-201b [a]	50	42	137 - 310	7.3	2.1	0.916	0.936
	Ots-208b [b]	50	52	158 - 342	9.9	5.5	0.943	0.954
	Ssa-408 [c]	50	32	184 - 308	4.0	3.1	0.827	0.934
Ots-N	*Ogo-2* [d]	60	19	202 - 256	4.5	0.4	0.756	0.854
	Ssa-197 [e]	60	38	181 - 318	11.9	0.4	0.915	0.940
Ots-O	*Ogo-4* [d]	56	17	132 - 164	15.6	1.6	0.776	0.884
	Ots-213 [b]	56	40	182 - 362	9.4	1.8	0.908	0.940
	Ots-G474 [f]	56	15	152 - 212	3.8	1.5	0.507	0.697
Ots-R	*Ots-3M* [g]	53	15	128 - 158	2.9	1.4	0.601	0.672
Ots-S	*Ots-9* [g]	60	8	99 - 113	5.0	1.0	0.668	0.709

[a] = Unpublished
[b] = Greig et al. 2003
[c] = Cairney et al. 2000
[d] = Olsen et al. 1998
[e] = Oreilly et al. 1996
[f] = Williamson et al. 2002
[g] = Banks et al. 1999

Table 3. Stock-of-origin assignments for five stocks of Chinook in the Yakima River Basin using GMA.

	American R.	Naches R.	upper Yakima R.	Marion Drain	lower Yakima R.
Jan - Feb	8.6%	18.2%	73.2%	0.0%	0.0%
March	0.0%	19.8%	80.3%	0.0%	0.0%
April	3.5%	24.0%	72.5%	0.0%	0.0%
May	5.7%	12.6%	77.8%	1.9%	2.0%
June-July	2.6%	0.0%	13.2%	11.9%	72.4%

Figure 1. Geographic location of the Chandler trap on the Yakima River, Washington and the primary streams in the basin.



Chapter 2

DNA-Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2011

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Abstract

We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of Chinook salmon spawners from the hatchery-control line (two generations of hatchery influence) and the supplementation hatchery line (SH – one generation of hatchery influence) in the Cle Elum experimental spawning channel for the 2010 brood year. The assignments were based on offspring genotypes at 14 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999999. Two thousand five hundred and fifty-nine of 2,920 fry from the 2010 brood that were genotyped at eight or more loci were assigned to a parental pair with 95% confidence. The number of progeny attributed to individual potential parents was quite variable, ranging from 0 to 333 for all males and from 0 to 165 for females. The sum of progeny attributed to the hatchery-control line males and females was 1,180, while the sum of progeny attributed to supplementation hatchery line males and females was 1,419.

Introduction

Although hatcheries have been extensively utilized in Chinook salmon management for over 100 years, only recently have rigorous experiments been developed to measure the relative reproductive success of hatchery- and natural-origin spawners in a shared natural setting. Some of the difficulty in designing informative studies has stemmed from the challenges of controlling entry to natural spawning areas and collecting representative samples of recently hatched fry. Furthermore, if control could be established over the potential spawners in the spawning area, the measurement of individual reproductive output still would require a means of associating individual fish captured in one year with individuals that spawned in a previous year. The spawning behavior of Chinook salmon adds to the complexity of quantifying individual reproductive output through behavioral observations: at a redd site, a female might be courted by several males that compete for access to the female, providing opportunities for multiple paternity in a single redd. In areas with moderate to high spawning densities, males might attend females on several adjacent redds. Microsatellites, a class of highly polymorphic, codominant DNA markers, provide a means to quantify individual spawners' reproductive output. A suite of 10 to 15 highly variable microsatellites can resolve individual identity in a moderate to large population, and through a simple inheritance model, can illuminate parent-offspring relationships.

Washington Department of Fish and Wildlife (WDFW) and the Yakama Nation (YN) are cooperating on a study of Chinook salmon reproductive success in a presumably closed access spawning observation channel at the Cle Elum Hatchery. Viewing blinds line the channel, allowing researchers to observe spawning activities.

Chinook salmon carrying visible external marks were released into the spawning channel in September 2010. Hatchery-control line (two generations of hatchery influence) males and females were released into three of six shared spawning areas and supplementation hatchery line (one generation of hatchery influence) males and females were released into the other three shared spawning areas to select and compete for mates. Prior to the release of the potential spawners, researchers collected and preserved samples of fin tissue to enable genetic characterization of the potential spawners and to allow subsequent inference of parent/offspring relationships after juveniles were collected and genotyped. One group of researchers examined morphological characteristics of these potential parents and observed and recorded spawning area behaviors and interactions. The results of the morphological and behavioral work are described in a separate report.

The potential parents' fin tissue samples and the collected progeny (fry) were delivered to the WDFW Molecular Genetics Laboratory in Olympia, Washington for genetic screening and parentage analysis following the same protocols that have been used from 2002 – 2007, 2009 – 2011 (Young and Kassler 2005, Kassler 2005, Kassler 2006, and Kassler and Von Bargen 2007, 2008, and 2010, Kassler et al. 2011). The genetic analyses provide direct, quantitative estimates of fry production by individual spawning Chinook salmon. This report presents the parentage results for the 2010 – 2011 Cle Elum spawning channel experiments.

Materials and Methods

Collection of potential spawners – 2010
Fin tissue was collected from a total of 48 adult females and 49 adult males (Table 1) prior to their release into each of six sections in the spawning channel during September 2010. The genetic analysis program CERVUS (version 3.0; Marshall et al. 1998) was used to check for identical multilocus genotypes among the potential parents. Data recorded for each released fish included gender, and whether it was of hatchery-control line origin or supplementation hatchery line origin (Table 1).

Collection of Fry
Fry collections occurred from December 2, 2010 to May 11, 2011. Fry samples were collected from each section daily when fry were present. During that period a total of 7,248 fry were collected.

DNA Extraction Methods
Genomic DNA was extracted by digesting a small piece of fin tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

PCR Methods
Potential spawners and offspring from 2011 were genotyped at 14 loci (Table 2). Potential spawners were screened twice and scored independently at all 14 loci by two biologists to minimize potential genotyping error of the parents.

The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units Go*Taq* Flexi DNA polymerase (Promega). Amplification was performed using MJ Research PTC-200 and AB 9700 thermocyclers. The thermal profile was as follows: an initial denaturation step of 2 minutes at 94oC; 40 cycles of 15 seconds at 94oC, 30 seconds at 49-58oC, and 1 minute at 72oC; plus a final extension step at 72oC for 10 minutes, followed by a final indefinite holding step at 4oC.

Microsatellite DNA loci (Table 2) were amplified via the polymerase chain reaction (PCR) using fluorescently labeled primers (obtained from Applied Biosystems or Integrated DNA Technologies). Loci were combined into multiplexes to increase efficiency and decrease costs.

Data were collected using an AB-3730 Genetic Analyzer. Applied Biosystems GENEMAPPER v.3.7 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et. al. 2007).

Parentage Assignments

The dataset included 42,328 single-locus genotypes. A genotyping error rate in that dataset of 1.0% would result in 423 incorrect single-locus genotypes. Our error rate is unknown, but possibly greater than 1%. Since parentage analyses involve comparing genotypes of candidate parental pairs with offspring genotypes, genotyping errors can produce parent-offspring genotype mismatches and suggest exclusion of true parent-offspring pairings from consideration. Alternatively, genotyping errors can lead to failure to exclude parent-offspring pairings that are incorrect. We used a maximum likelihood procedure, implemented in CERVUS (version 3.0; Marshall et al. 1998) to infer parent-offspring relationships. The procedure uses allele frequency data to assign likelihoods to parent-offspring combinations, and allows mismatching genotypic data to be evaluated concurrently with matching genotype data.

Genotyping error is not the only potential source of mismatches between the genotypes of fry and their putative parents. We would expect allele misidentification to be randomly distributed throughout the genotype dataset and not to occur in clusters. Parent-offspring mismatches can result also from germ-line mutation in which a parent passes a changed allele to its offspring or from the inadvertent exclusion of one or more contributing parents from the parental dataset. These mismatches are due to correctly assigned but unexpected genotypes, and we expect that those genotypes should cluster in families. Distinguishing between mutation-based mismatches and mismatches that result from reproductive participation by un-genotyped parents is difficult. Assuming that all dams in the experimental channel are represented in the parental data set, we might suspect reproductive participation by one or more unrepresented sires if groups of fry that are assigned to a dam-offspring relationship with no mismatching loci, have multiple locus mismatches with all candidate sires, and no more than four alleles at a locus within the group. The data set was carefully examined for evidence of reproductive contributions by such un-genotyped parents (because evidence of ungenotyped parents had been observed in previous years).

Results

Parents

Genetic analysis revealed that all 97 fish released or found in the spawning channel had unique genotypes. There were a total of 25 hatchery control line (HC) adult males, 24 HC adult females, 24 supplementation hatchery line (SH) adult males, and 24 SH adult females. Four HC males and four HC females were released into three of the six sections and four SH males and four SH females were released into the other three sections (Table 1).

Loci Screened

A total of 14 loci were screened and all 14 were used in the analysis (Table 2). Number of alleles ranged from 5 - 32 (*Ots-9* and *Omm-1080* respectively) and observed heterozygosity ranged from 0.330 – 0.958 (*Ots-G474* and *Omm-1080* respectively). Individual exclusionary power was below 46.3% for five loci (*Ogo-2*, *Ogo-4*, *Ots-G474*, *Ots-3M*, and *Ots-9*) and above 60.5% for the remaining loci when neither parent was known. Exclusionary power was below 40.6% for three loci (*Ots-G474*, *Ots-3M* and *Ots-9*) and above 59.2% for the remaining loci when one parent was known. Cumulative exclusionary power was 1.000000 for analysis using all loci when one parent was known.

Parentage Assignments

Parentage assignments were made when genotype data was available for nine or more loci. All 97 parents were genotyped at 12 or more loci while 2,920 of the 3,000 offspring were successfully genotyped at eight or more loci (Table 3).

Parentage analysis was conducted independently for each of the six sections using all 97 adults as possible parents. Each fry was assigned a dam-sire-fry combinations (trios) based on the most likely candidate male parents (sires) and female parents (dams). Those assignments yielded possible. Any fry-sire assignments with more than two mismatching loci were excluded from further consideration.

Of the total 2,920 fry included in the analysis a total of 2,599 fry were assigned to a single male and female parent (2,599/2,920 = 89.0%; Table 4).

Discussion

Eighty-nine percent successes were achieved at inferring parent-offspring relationships. Examination of Table 5 reveals a very uneven pattern of reproductive success among the candidate parents. Based on the subsample of 2,599 fry that were successfully assigned parents, the range of inferred reproductive output among males was 0 - 333 fry; the range for the same period in reproductive output among females was 0 - 165 fry. Some of the dam-sire matings we inferred are well supported (there were a lot of fry assigned to them) and some are weakly supported (not many fry were assigned to them). Caution should be used when interpreting dam-sire-fry combinations that were inferred rarely. Future integration of fecundity estimates for spawners will enrich the interpretation of these estimates of reproductive output.

Interpretation of the inferred parental reproductive output based on parentage assignments by genetic analysis requires the consideration and analysis of individual fish attributes, including fecundity and body size, the closed nature of the experimental environment in which sub-dominant males had a more limited number of alternative females to court than they might have had in an open system, and relative stocking levels and synchronicity of spawning.

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Table 1. Potential Chinook salmon spawners in the six section of the Cle Elum experimental spawning channel in 2010. Origin is identified as hatchery-control (HC) or supplementation hatchery (SH).

	Section 1 – 1A	Section 1 – 2A	Section 1 – 3A	Section 2 – 1A	Section 2 – 2A	Section 2 – 3A
Origin	**Females**	**Females**	**Females**	**Females**	**Females**	**Females**
SH	4	--	4	--	4	--
HC	--	4	--	4	--	4
	Males	**Males**	**Males**	**Males**	**Males**	**Males**
SH	4	--	4	--	4	--
HC	--	5	--	4	--	4

	Section 1 – 1B	Section 1 – 2B	Section 1 – 3B	Section 2 – 1B	Section 2 – 2B	Section 2 – 3B
Origin	**Females**	**Females**	**Females**	**Females**	**Females**	**Females**
SH	4	--	4	--	4	--
HC	--	4	--	4	--	4
	Males	**Males**	**Males**	**Males**	**Males**	**Males**
SH	4	--	4	--	4	--
HC	--	4	--	4	--	4

Table 2. Locus summary.

Locus	# alleles	# parents genotyped	H_O (observed)	HE (expected)	Exclusionary power	
					neither parent	one parent
Oki-100	20	97	0.948	0.916	0.698	0.822
Ots-201b	19	97	0.918	0.878	0.605	0.755
Ots-208b	26	97	0.938	0.936	0.757	0.861
Ssa-408	18	95	0.674	0.918	0.701	0.825
Ogo-2	9	97	0.753	0.820	0.463	0.638
Ssa-197	18	97	0.866	0.909	0.674	0.806
Ogo-4	10	97	0.773	0.785	0.412	0.592
Ots-213	20	97	0.938	0.919	0.706	0.828
Ots-G474	6	97	0.330	0.327	0.055	0.180
Omm-1080	32	95	0.958	0.954	0.815	0.898
Ots-3M	7	97	0.639	0.630	0.230	0.406
Ots-211	23	97	0.907	0.932	0.743	0.853
Ots-212	19	97	0.928	0.884	0.610	0.758
Ots-9	5	97	0.711	0.665	0.240	0.396

Table 3. Summary of genotyping efficiency in potential parents and offspring.

Loci genotyped	Parents (10IR)	Offspring (11HX)
14	94	1,906
13	2	366
12	1	405
11	0	167
10	0	36
9	0	22
8	0	18
7	0	8
6	0	7
5	0	11
4	0	7
3	0	7
2	0	8
1	0	2
0	0	30
	97	3,000

Table 4. Parentage among spawning pairs from each of the six sections inferred with 95% confidence for Chinook salmon fry spawned in fall of 2010. The row and column headers describe the adult males, females, the origin, and section where each male and female were released. "N" for males indicates total number of progeny assigned to this male, "N" for females indicates a subtotal of the number of progeny assigned to this female (totals can be obtained by summing female "N" across pages [see Table 5 for totals]). The section labels identifies the section where fry were collected. Boxes indicate groups of parents that were introduced into the same section.

Fry recovered in Section 1 - 1			**Male**	10IR001	10IR006	10IR007	10IR009	10IR013	10IR055	10IR056	10IR058
	Origin			SH	SH	SH	SH	SH	SH	SH	SH
		Section		1-1A	1-1A	1-1A	1-1A	1-1B	1-1B	1-1B	1-1B
Female			**N =**	0	86	91	0	52	140	34	21
10IR002	SH	1-1A	46			46					
10IR003	SH	1-1A	86		86						
10IR004	SH	1-1A	45			45					
10IR005	SH	1-1A	0								
10IR008	SH	1-1B	51					16	14		21
10IR018	SH	1-1B	100						100		
10IR043	SH	1-1B	1							1	
10IR054	SH	1-1B	95					36	26	33	

Table 4 continued.

Fry recovered in Section 1 - 2			Male	10IR006	10IR007	10IR013	10IR055	10IR056	10IR058	10IR012	10IR015	10IR016	10IR017	10IR038	10IR059	10IR062	10IR064	10IR065
	Origin			SH	SH	SH	SH	SH	SH	HC	HC	HC	HC	HC	HC	HC	HC	HC
		Section		1-1A	1-1A	1-1B	1-1B	1-1B	1-1B	1-2A	1-2A	1-2A	1-2A	1-2A	1-2B	1-2B	1-2B	1-2B
Female			N =	7	17	13	16	4	1	0	134	0	6	2	26	148	0	66
10IR002	SH	1-1A	13		13													
10IR003	SH	1-1A	7	7														
10IR004	SH	1-1A	4		4													
10IR008	SH	1-1B	6			5			1									
10IR018	SH	1-1B	8				8											
10IR043	SH	1-1B	1				1											
10IR054	SH	1-1B	19			8	7	4										
10IR010	HC	1-2A	0															
10IR011	HC	1-2A	38								36			2				
10IR014	HC	1-2A	53								53							
10IR019	HC	1-2A	51								45		6					
10IR057	HC	1-2B	15												15			
10IR060	HC	1-2B	122													89		33
10IR061	HC	1-2B	23												10			13
10IR063	HC	1-2B	80												1	59		20

Table 4 continued.

Fry recovered in Section 1 - 3	Origin	Section	Male	10IR006	10IR007	10IR013	10IR055	10IR056	10IR015	10IR059	10IR062	10IR065	10IR020	10IR022	10IR023	10IR026	10IR070	10IR071	10IR072	10IR073
	Origin			SH	SH	SH	SH	SH	HC	HC	HC	HC	SH	SH	SH	SH	SH	SH	SH	SH
		Section		1-1A	1-1A	1-1B	1-1B	1-1B	1-2A	1-2B	1-2B	1-2B	1-3A	1-3A	1-3A	1-3A	1-3B	1-3B	1-3B	1-3B
Female			N =	6	4	3	5	2	53	4	20	10	17	98	0	0	2	244	0	0
10IR002	SH	1-1A	3		3															
10IR003	SH	1-1A	6	6																
10IR004	SH	1-1A	1		1															
10IR018	SH	1-1B	2				2													
10IR054	SH	1-1B	8			3	3	2												
10IR011	HC	1-2A	6						6											
10IR014	HC	1-2A	41						41											
10IR019	HC	1-2A	6						6											
10IR057	HC	1-2B	4							4										
10IR060	HC	1-2B	21								15	6								
10IR061	HC	1-2B	3									3								
10IR063	HC	1-2B	6								5	1								
10IR021	SH	1-3A	13										13							
10IR024	SH	1-3A	52										4	48						
10IR025	SH	1-3A	0																	
10IR027	SH	1-3A	50											50						
10IR066	SH	1-3B	43															43		
10IR067	SH	1-3B	15															15		
10IR068	SH	1-3B	77														2	75		
10IR069	SH	1-3B	111															111		

Table 4 continued.

Fry recovered in Section 2 - 1			Male	10IR006	10IR007	10IR013	10IR055	10IR056	10IR015	10IR017	10IR059	10IR062	10IR065	10IR020	10IR022	10IR070	10IR071	10IR029	10IR031	10IR033	10IR035	10IR076	10IR077	10IR080	10IR081
	Origin			SH	SH	SH	SH	SH	HC	HC	HC	HC	HC	SH	SH	SH	SH	HC	HC	HC	HC	HC	HC	HC	HC
		Section		1-1A	1-1A	1-1B	1-1B	1-1B	1-2A	1-2A	1-2B	1-2B	1-2B	1-3A	1-3A	1-3B	1-3B	2-1A	2-1A	2-1A	2-1A	2-1B	2-1B	2-1B	2-1B
Female			N =	1	1	2	2	2	27	2	5	15	7	11	40	1	67	129	0	23	0	0	0	0	100
10IR002	SH	1-1A	1		1																				
10IR003	SH	1-1A	1	1																					
10IR008	SH	1-1B	1			1																			
10IR018	SH	1-1B	2				2																		
10IR054	SH	1-1B	3			1		2																	
10IR011	HC	1-2A	10						10																
10IR014	HC	1-2A	14						14																
10IR019	HC	1-2A	5						3	2															
10IR060	HC	1-2B	15									10	5												
10IR061	HC	1-2B	5								4		1												
10IR063	HC	1-2B	7								1	5	1												
10IR021	SH	1-3A	11											11											
10IR024	SH	1-3A	20												20										
10IR027	SH	1-3A	20												20										
10IR066	SH	1-3B	9														9								
10IR067	SH	1-3B	6														6								
10IR068	SH	1-3B	27													1	26								
10IR069	SH	1-3B	26														26								
10IR028	HC	2-1A	60															37		23					
10IR030	HC	2-1A	50															50							
10IR032	HC	2-1A	42															42							
10IR034	HC	2-1A	0																						
10IR074	HC	2-1B	35																						35
10IR075	HC	2-1B	12																						12
10IR078	HC	2-1B	39																						39
10IR079	HC	2-1B	14																						14

Table 4 continued.

Fry recovered in Section 2 - 2			Male	10IR007	10IR013	10IR015	10IR059	10IR062	10IR020	10IR022	10IR071	10IR029	10IR033	10IR081	10IR036	10IR044	10IR045	10IR089	10IR039	10IR084	10IR085	10IR086
	Origin			SH	SH	HC	HC	HC	SH	SH	SH	HC	HC	HC	SH	SH	SH	SH	SH	SH	SH	SH
		Section		1-1A	1-1B	1-2A	1-2B	1-2B	1-3A	1-3A	1-3B	2-1A	2-1A	2-1B	2-2A	2-2A	2-2A	2-2A	2-2B	2-2B	2-2B	2-2B
Female			N =	1	1	5	3	5	1	8	11	25	4	46	0	185	19	9	0	10	0	71
10IR004	SH	1-1A	1	1																		
10IR008	SH	1-1B	1		1																	
10IR011	HC	1-2A	2			2																
10IR014	HC	1-2A	3			3																
10IR057	HC	1-2B	2				2															
10IR060	HC	1-2B	3					3														
10IR061	HC	1-2B	1				1															
10IR063	HC	1-2B	2					2														
10IR021	SH	1-3A	1						1													
10IR024	SH	1-3A	5							5												
10IR027	SH	1-3A	3							3												
10IR066	SH	1-3B	2								2											
10IR068	SH	1-3B	1								1											
10IR069	SH	1-3B	8								8											
10IR028	HC	2-1A	7									3	4									
10IR030	HC	2-1A	6									6										
10IR032	HC	2-1A	16									16										
10IR074	HC	2-1B	19											19								
10IR075	HC	2-1B	6											6								
10IR078	HC	2-1B	11											11								
10IR079	HC	2-1B	10											10								
10IR037	SH	2-2A	65													48	17					
10IR042	SH	2-2A	18													9		9				
10IR087	SH	2-2A	84													84						
10IR088	SH	2-2A	46													44	2					
10IR040	SH	2-2B	18																			18
10IR041	SH	2-2B	32																			32
10IR082	SH	2-2B	10																	10		
10IR083	SH	2-2B	21																			21

Table 4 continued.

Fry recovered in Section 2 - 3	Origin	Section	Male	10IR007	10IR055	10IR015	10IR038	10IR062	10IR065	10IR020	10IR022	10IR070	10IR071	10IR029	10IR081	10IR044	10IR089	10IR084	10IR086	10IR047	10IR049	10IR051	10IR053	10IR093	10IR094	10IR095	10IR097
				SH	SH	HC	HC	HC	HC	SH	SH	SH	SH	HC	HC	SH	SH	SH	SH	HC	HC	HC	HC	HC	HC	HC	HC
				1-1A	1-1B	1-2A	1-2A	1-2B	1-2B	1-3A	1-3A	1-3B	1-3B	2-1A	2-1B	2-2A	2-2A	2-2B	2-2B	2-3A	2-3A	2-3A	2-3A	2-3B	2-3B	2-3B	2-3B
Female			N =	1	6	1	1	5	2	3	6	1	11	5	21	45	5	3	32	0	2	20	218	0	12	308	0
10IR004	SH	1-1A	1	1																							
10IR018	SH	1-1B	4		4																						
10IR054	SH	1-1B	2		2																						
10IR011	HC	1-2A	1				1																				
10IR019	HC	1-2A	1			1																					
10IR060	HC	1-2B	4					3	1																		
10IR063	HC	1-2B	3					2	1																		
10IR021	SH	1-3A	3							3																	
10IR024	SH	1-3A	2								2																
10IR027	SH	1-3A	4								4																
10IR066	SH	1-3B	2										2														
10IR067	SH	1-3B	2										2														
10IR068	SH	1-3B	4									1	3														
10IR069	SH	1-3B	4										4														
10IR028	HC	2-1A	1											1													
10IR030	HC	2-1A	2											2													
10IR032	HC	2-1A	2											2													
10IR074	HC	2-1B	8												8												
10IR075	HC	2-1B	2												2												
10IR078	HC	2-1B	6												6												
10IR079	HC	2-1B	5												5												
10IR037	SH	2-2A	7													7											
10IR042	SH	2-2A	10													5	5										
10IR087	SH	2-2A	27													27											
10IR088	SH	2-2A	6													6											
10IR040	SH	2-2B	14																14								
10IR041	SH	2-2B	11																11								
10IR082	SH	2-2B	3															3									
10IR083	SH	2-2B	7																7								

Table 4 continued.

Fry recovered in Section 2 - 3			Male	10IR007	10IR055	10IR015	10IR038	10IR062	10IR065	10IR020	10IR022	10IR070	10IR071	10IR029	10IR081	10IR044	10IR089	10IR084	10IR086	10IR047	10IR049	10IR051	10IR053	10IR093	10IR094	10IR095	10IR097
	Origin			SH	SH	HC	HC	HC	HC	SH	SH	SH	SH	HC	HC	SH	SH	SH	SH	HC	HC	HC	HC	HC	HC	HC	HC
		Section		1-1A	1-1B	1-2A	1-2A	1-2B	1-2B	1-3A	1-3A	1-3B	1-3B	2-1A	2-1B	2-2A	2-2A	2-2B	2-2B	2-3A	2-3A	2-3A	2-3A	2-3B	2-3B	2-3B	2-3B
Female			N =	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	109	0	6	154	0
10IR046	HC	2-3A	52																		1	8	43				
10IR048	HC	2-3A	17																			2	15				
10IR050	HC	2-3A	23																				23				
10IR052	HC	2-3A	28																				28				
10IR090	HC	2-3B	6																						6		
10IR091	HC	2-3B	7																							7	
10IR092	HC	2-3B	52																							52	
10IR096	HC	2-3B	95																							95	

Table 5. Total number of offspring assigned to females and males from each of the six sections in the spawning channel and the life stage (HC - hatchery control line; SH - supplementation hatchery line) for each fish.

Females	Section	HC or SH	Total Offspring	Males	Section	HC or SH	Total Offspring
10IR002	1-1A	SH	63	10IR001	1-1A	SH	0
10IR003	1-1A	SH	100	10IR006	1-1A	SH	100
10IR004	1-1A	SH	52	10IR007	1-1A	SH	115
10IR005	1-1A	SH	0	10IR009	1-1A	SH	0
10IR008	1-1B	SH	59	10IR013	1-1B	SH	71
10IR018	1-1B	SH	116	10IR055	1-1B	SH	169
10IR043	1-1B	SH	2	10IR056	1-1B	SH	42
10IR054	1-1B	SH	127	10IR058	1-1B	SH	22
10IR010	1-2A	HC	0	10IR012	1-2A	HC	0
10IR011	1-2A	HC	57	10IR015	1-2A	HC	220
10IR014	1-2A	HC	111	10IR016	1-2A	HC	0
10IR019	1-2A	HC	63	10IR017	1-2A	HC	8
10IR057	1-2B	HC	21	10IR038	1-2A	HC	3
10IR060	1-2B	HC	165	10IR059	1-2B	HC	38
10IR061	1-2B	HC	32	10IR062	1-2B	HC	193
10IR063	1-2B	HC	98	10IR064	1-2B	HC	0
				10IR065	1-2B	HC	85
10IR021	1-3A	SH	28	10IR020	1-3A	SH	32
10IR024	1-3A	SH	79	10IR022	1-3A	SH	152
10IR025	1-3A	SH	0	10IR023	1-3A	SH	0
10IR027	1-3A	SH	77	10IR026	1-3A	SH	0
10IR066	1-3B	SH	56	10IR070	1-3B	SH	4
10IR067	1-3B	SH	23	10IR071	1-3B	SH	333
10IR068	1-3B	SH	109	10IR072	1-3B	SH	0
10IR069	1-3B	SH	149	10IR073	1-3B	SH	0
10IR028	2-1A	HC	68	10IR029	2-1A	HC	159
10IR030	2-1A	HC	58	10IR031	2-1A	HC	0
10IR032	2-1A	HC	60	10IR033	2-1A	HC	27
10IR034	2-1A	HC	0	10IR035	2-1A	HC	0
10IR074	2-1B	HC	62	10IR076	2-1B	HC	0
10IR075	2-1B	HC	20	10IR077	2-1B	HC	0
10IR078	2-1B	HC	56	10IR080	2-1B	HC	0
10IR079	2-1B	HC	29	10IR081	2-1B	HC	167
10IR037	2-2A	SH	72	10IR036	2-2A	SH	0
10IR042	2-2A	SH	28	10IR044	2-2A	SH	230
10IR087	2-2A	SH	111	10IR045	2-2A	SH	19
10IR088	2-2A	SH	52	10IR089	2-2A	SH	14
10IR040	2-2B	SH	32	10IR039	2-2B	SH	0
10IR041	2-2B	SH	43	10IR084	2-2B	SH	13
10IR082	2-2B	SH	13	10IR085	2-2B	SH	0
10IR083	2-2B	SH	28	10IR086	2-2B	SH	103
10IR046	2-3A	HC	52	10IR047	2-3A	HC	0
10IR048	2-3A	HC	17	10IR049	2-3A	HC	1
10IR050	2-3A	HC	23	10IR051	2-3A	HC	10
10IR052	2-3A	HC	28	10IR053	2-3A	HC	109
10IR090	2-3B	HC	6	10IR093	2-3B	HC	0
10IR091	2-3B	HC	7	10IR094	2-3B	HC	6
10IR092	2-3B	HC	52	10IR095	2-3B	HC	154
10IR096	2-3B	HC	95	10IR097	2-3B	HC	0
			2,599				2,599