Report

Genetic Analysis for Spring-run San Joaquin River Chinook Salmon

Annual Technical Report

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Annual Report –Year 2018 Activities

San Joaquin River Restoration Program (SJRRP)

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I. Summary

Through Interagency Agreement R14PG00097, the NOAA Southwest Fisheries Science Center (SWFSC) collected genotypic data from Chinook salmon and performed genetic analyses to inform the actions of the Salmon Conservation and Research Facility (SCARF) and other relevant components of the San Joaquin River Restoration Program (SJRRP). The following were specified as deliverables of the genetic monitoring and management program, intended to minimize genetic impacts on donor stock populations and augment the long-term sustainability of the San Joaquin River salmon populations:

- Annual sex identification for the Salmon Conservation and Research Facility (SCARF) broodstock;
- Broodstock family reconstruction and creation of spawner candidate list based on relatedness for the SCARF;
- Establishment of Parentage Based Tagging (PBT) program for the San Joaquin River Chinook salmon population;
- Parentage inference for San Joaquin River young of year juvenile and returning adult salmon;
- Population genetics analysis for the San Joaquin River population;
- Annual and summary reporting;
- Raw genotype data acquired through the agreement.

This report summarizes our findings for the first year of the current agreement and the fifth year of the project (2018). Data deliverables can be found in the files referenced in curly brackets.

II. Genetic sex identification of the SCARF spring-run broodstock [2.1]

A key component to limiting the incidence of precocious male maturation at the SCARF is to identify the genetic sex of Chinook salmon in their first year and restrict the feeding rates of males. We received samples from juvenile Chinook salmon representing San Joaquin broodyear (SJBY) 2017 (N=1,962). As the Feather River Hatchery was unable to reach their own spring-run egg take goals this year, these fish were instead sampled from two groups: (1) siblings of SJBY2016 broodstock that were being held as ”translocation fish”; and (2) SCARF 2016/2017 production fish, slated for release to the river. DNA was extracted and individuals were genotyped with the OtY3 marker, on the y-chromosome possessed only by males, to determine sex.

Results: For SJBY2017, we genotyped 1,962 juvenile Chinook salmon and identified 1,024 males and 931 females. For seven of the sampled individuals, gender was ambiguous or undetermined, and they will be re-genotyped. This information was provided to SCARF staff so that males could be segregated and placed on an alternative feeding regime. {SJ_Spring_SCARFbroodstock_2017metagenos.xlsx}

III. Family reconstruction analysis of spring-run broodstock samples [Task 3.1]

For the first five years of this project (2012-2016), San Joaquin SCARF broodstock was obtained annually from the Feather River Hatchery (FRH) and the relationships among the contributing adult individuals determined using parentage (Table 1). This information can inform our estimates of effective population size (Ne) for SCARF broodstock as the presence of full- or half-siblings among the contributing FRH adults will depress Ne in the resulting population. As described above, in 2017, there were not sufficient returning spring-run adults at the FRH to support broodstock collection for the San Joaquin program, so two alternative groups of fish were used to provide broodstock for 2017. Fortunately, the spring-run returns at the Feather River Hatchery in 2018 were large enough to allow the collection of eggs from ~370 crosses. We calculated basic population genetic parameters and identified the relationships among these contributing adults using parentage.

Results: For the FRH adults contributing SCARF broodstock in 2018, we were able to successfully determine familial relationships for ~92% (Table 1). Among these 654 FRH SCARF ‘founders’ with known parentage, we identified 143 full sibships ranging in size from 2-13 members (i.e., a contributing FRH parent had brothers/sisters that were also contributing parents), while the remaining 209 individuals were putatively unrelated (i.e., not full-sibs). We note that 2018 was the first year since 2012 that collecting broodstock from a larger number of FRH parents increased the proportion of parents with at least one full sibling also contributing, but did not increase the number of parents with no siblings. This is not expected to be a problem as there are still a large proportion of unrelated broodstock individuals and there are plenty of opportunities to equalize family size before these fish mature and are spawned.

Measures of heterozygosity and the proportion of polymorphic loci for the annual broodstock has remained stable over the years of collection and is similar to what is observed in the overall FRH spring- run broodstock.

Table 1 Distribution of full siblings among FRH adults used to found SJRRP broodstock each year from 2012-2018 (2017 absent). Shown are the total number of adults successfully genotyped (Ntot), unbiased expected heterozygosity (HZ), observed heterozygosity (HO), the proportion of polymorphic loci (Lpoly), the number of adults successfully assigned (Nassn), the number and size of observed full sibships, the number of adults unrelated to other adults in the same year (Nun) and the proportion of individuals with one or more full sibling that also contributed to the SCARF broodstock (%FS) in that year.

FRH Spawn						Full sibships of size:											
Year	N_{tot}	H_Z	H_O	L_{poly}	N_{assn}	13	10	9	8	7	6	5	4	3	2	N_{un}	%FS
2012	140	0.371	0.365	0.98	132	0	0	0	0	0	0	0	1	3	18	88	0.37
2013	182	0.372	0.364	0.98	146	0	0	0	0	0	0	0	0	2	17	106	0.27
2014	213	0.372	0.378	0.97	182	0	0	0	0	0	0	1	0	7	18	120	0.34
2015	735	0.368	0.372	0.98	668	0	0	0	0	3	3	6	12	30	89	283	0.58
2016	718	0.369	0.367	0.97	586	0	0	0	1	0	0	4	10	23	81	287	0.51
2017	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2018	712	0.372	0.373	1	654	1	2	1	2	2	4	5	16	40	70	209	0.68

IV. Pairwise relatedness matrix [Task 3.2]

The spawning of SCARF individuals for SJRRP reintroductions is guided by a relatedness mating 'matrix'. Generally, for each female spawner, we use genetic information to rank each potential male mate on the basis of increasing relatedness (least related to most related for each female). During spawning at the SCARF, hatchery personnel use the matrix to attempt to select the least related male(s) for each female. Because of asynchrony of maturation, it is not possible to spawn every female with the least related males. In addition, the relatedness estimator used (rxy of Queller & Goodnight, 1989) is unbiased, but has a large variance, so small differences in the values are generally not meaningful.

Results: The year 2018 was the fourth year for which SCARF captive broodstock individuals (collected as juvenile offspring of FRH adults from 2012-2016) were themselves maturing and ready to be spawned. We received a list of 294 ripe fish representing SJBY2012 (1 female), SJBY2013 (18 males/8 females), SJBY2014 (35 males/33 females), SJBY2015 (112 males/60 females), and SJBY2016 (26 males/1 female. Utilizing the same genotype data employed for identifying parentage, we calculated the pairwise relatedness between all possible pairs of males and females. These values ranged from -1 to 1 (unbiased), with all values <0 essentially indicating that a pair of individuals are unrelated. We then ranked all males for each female based on their calculated rxy with that female. This mating matrix was provided to SCARF staff and utilized to inform mate choice during the 2018 spawn season.

{SCARF_breeding_matrix_2018_final.xlsx}

V. Implementation of parentage based tagging (PBT) for the San Joaquin Chinook salmon population [Task 4]

With the cessation of the fall-run Trap & Haul program in 2017, the spring-run Chinook salmon raised at the SCARF (normally derived from the Feather River Hatchery spring-run broodstock) are now the primary stock in the San Joaquin River that will be genotyped in order to tag their offspring using parentage (as described above in section II). As adults (raised in captivity), SCARF broodstock can either be released directly to the river to voluntarily choose mates and spawn in the wild (adult-release program, see section VI) or they can be spawned in the hatchery (using the mating matrix described in section IV) and their resulting offspring released to the San Joaquin River. Whether produced in the river or at the hatchery, when the offspring of SCARF broodstock are trapped following release or return as adults, they will be identified as program fish by assigning them back to their parents. In addition to tagging these individuals as SJRRP spring broodstock, assigning these offspring back to their FRH parents provides two additional pieces of information: the relative reproductive success of each mating (i.e., how many offspring each parent pair contributes to the next generation), and the true relationship among some groups of offspring (i.e. full siblings). This information can be used to equalize family sizes (and stabilize Ne) when space or other constraints dictate that the number of broodstock fish needs to be reduced, which was the case in 2018.

Results: For each SCARF broodyear, we calculated the same genetic diversity statistics as appear in Table 1 for the parent collections from the FRH. Table 2 summarizes these estimates of polymorphism. Recall that SJBY2017 was very different from previous broodyears, as no broodstock were available from the Feather River Hatchery. Therefore, SCARF SJBY2017 broodstock was instead derived from the siblings of SJBY2016 broodstock and SCARF production fish from 2016 and 2017, which were spawned using the mating matrix. Sibship analysis was used to select 2016 FRH sibling families that were not represented or were underrepresented in SCARF SJBY2016 broodstock {SJ_BY2016sibs_fullsibs.xlsx}. Initially, 1,962 fish were chosen to comprise BY2017; however, over half of these fish were subsequently released to the river due to space constraints at the SCARF. For the initial collection of SJBY2017 fish (n=1,962), we importantly note no significant reductions in heterozygosity compared to previous years, nor do we see any chronic deficits in observed heterozygosity, which would indicate above average inbreeding. In fact, we see an increase in observed heterozygosity compared to expected heterozygosity, which may reflect the more diverse cohort representation in this broodyear; increases in observed heterozygosity can indicate that a sample is composed of a mixture. While these groups are not expected to be very different (FRH offspring + F1 of FRH offspring), the mating matrix is intended to avoid matings between closely related individuals, thereby retaining a maximum amount of genetic diversity. This is likely a good indication that program goals are being met or exceeded with respect to a genetically diverse and representative broodstock.

Table 2 also shows the observed variance in family size among SJBY2012-SJBY2017 spring-run broodstock fish. For the full collection of SJBY2017, we identified full- and half-sibling families, which ranged in size from 1-35 members, and used this information to provide recommendations for the number of individuals that could be released from each family {SJ_BY2017_sibships.xlsx}. The full accounting of which fish remain will be filled in upon the next full accounting of SJBY2017.

Similarly, the final expected number of assignments was unavailable for SJBY2017, since fish were not taken from the FRH and exactly which fish remain in SJBY2017 has not been determined; however, a 97.5% rate of assignment for the full collection is comparable to that observed in previous years and demonstrates the power and efficiency of parentage-based tagging (PBT).

Table 2 For each SCARF broodyear, we report the number of individuals successfully genotyped (Ntot), unbiased expected heterozygosity (HZ), observed heterozygosity (HO), the proportion of polymorphic loci (Lpoly), the number of fish assigned back to parents (Nassn), the expected number of fish assigned to parents and the sizes of full-sibling families found within four years of SJRRP/SCARF spring-run broodstock. * See the text for an explanation of BY2017.

SJRRP						Exp.	Number of full-sib families of size:									
BY	N_{tot}	H_Z	H_O	L_{poly}	N_{assn}	N_{assn}	1	2	3	4	5	6	7	8	9	10
BY2012	382	0.369	0.364	0.97	373	372	5	7	4	15	14	16	12	4	0	0
BY2013	405	0.37	0.372	0.97	343	351	5	7	1	5	5	9	16	7	6	0
BY2014	440	0.371	0.372	0.97	402	418	6	6	5	19	29	20	4	0	0	0
BY2015	1375	0.369	0.367	0.98	1344	1241	111	72	61	60	34	31	18	11	4	6
BY2016	464	0.365	0.362	0.97	442	447	129	83	36	7	1	1	0	0	0	0
BY2017*	1962	0.372	0.392	0.98	1913	n/a	-	-	-	-	-	-	-	-	-	-

Our ability to identify full-sibling families was particularly useful in 2018, wherein construction delays of the permanent hatchery facility led to severe space constraints at the interim SCARF facility for adults as well as juveniles. This required the release of some captive broodstock adults to the river, which was guided by knowledge of family structure. The detailed list of full-sibling groups was provided to the SCARF staff together with family-specific release recommendations, which were used to further equalize family sizes in the retained broodstock adults. {SJ_potential_spawn_release_2018revised_fullsibs.xlsx}

VI. Parentage inference for returning adult and young of year juvenile Chinook salmon in the San Joaquin River [Task 5]

In 2016, 2017 and 2018, the offspring of the first three years of SCARF broodstock spawning (described in section IV) were released to the river as juveniles. Since we have genotyped all of their parents, 100% of these offspring carry genetic tags. When these fish are encountered either as juveniles further down in the Sacramento/San Joaquin system (e.g., at the salvage facilities) or as returning adults in subsequent years, we will be able to unambiguously identify them as offspring of SCARF spring-run broodstock.

While we did not receive any samples from natural adult returns to the San Joaquin River in 2018, the PBT databases are in place to identify offspring of program fish when they do return to the river.

In 2018, spring-run SCARF broodstock adults were also released into the San Joaquin River below Friant Dam as they were maturing. Thirteen redds were documented and 817 juveniles were subsequently trapped and sampled downstream in rotary screw traps (RST). Genetic analysis of these juveniles yielded a variety of insights:

1) A matching-samples analysis revealed that 21 fish had been sampled twice, occasionally at the same site, but more commonly at a downstream location. Using the sample dates, we were able to calculate transit time between the two sample locations, which ranged from 1-144 days. Two fish apparently migrated upstream.

2) A subset of juvenile fish was labeled yearlings (n=19) by the collectors, presumably because of their larger size. Twelve of these yearlings were from a single full-sibling family that assigned to two fall-run trap-and-haul parents from 2016. Three other yearlings also assigned to fall-run trap- and-haul parents. An additional three yearlings were spring-run SCARF production fish from trap efficiency tests or juvenile releases. The final yearling was unassigned.
3) A total of 84 juveniles assigned with both parents as adult-release spring-run, representing 16 distinct pairings and a total of 11 unique females. While it was encouraging that natural spawning had taken place, the expectation was that ALL of the juveniles would fall into this category (offspring of adult releases). This necessitated additional analyses to track down the origin of the unassigned juveniles.
4) A total of 368 juveniles were found to be SCARF production fish (offspring of program broodstock). The production fish were apparently used to generate trap efficiency estimates and should have been identified as such and not sampled. Production fish identified here may also been from unintentional releases or have been holdovers from intentional juvenile releases.
5) We identified 79 juveniles that appeared to be the offspring of an adult-release male and an unknown/unsampled female. These were distributed into 17 full-sibling families and represented eight unique male parents, two of which were also represented in a family from 2) above. We also identified 35 juveniles that appeared to be the offspring of an adult-release female and an unknown/unsampled male. These were distributed into 14 full-sibling families and represented 11 unique female parents, nine of which were also represented in a family from 2) above. We hypothesized that some adult Chinook salmon may have migrated upstream through the Eastside/Chowchilla bypass during high flows and spawned with the adult-release fish.

6) And finally, 226 juveniles captured in rotary screw traps in 2018 remain unidentified. Clustering and GSI analyses indicated that all of these fish originated from a Central Valley lineage and did not appear to be FRH or SCARF fish. Our best hypothesis for their origin is that these may have been Salmon in the Classroom fish, likely from the Merced River hatchery, that were released to the river. They may also be offspring of fish that migrated through the Eastside/Chowchilla bypass undocumented and spawned in the Restoration Area.

   {SJ_RST2018_complete_analysis.xlsx}

VII. Data Management [Task 6]

Data are currently being managed and archived locally and are also attached to this report. When we receive direction as to the format and final destination for these data, we will formally submit or upload them. In the interim, any additional data or analyses are available upon request from the authors.

VIII. Annual Report [Task 7]

This document, submitted 7/30/2019.