

March 30, 2004

To: Tricia Parker
Habitat Restoration Coordinator/Fishery Biologist
Anadromous Fish Restoration Program
Red Bluff Fish and Wildlife Office
10950 Tyler Road, Red Bluff, CA 96080
PH: (530) 527-3043 x254
FAX: (530) 529-0292

RE: Second quarter status report for the project "Contaminant-induced sex-reversal of Fall-run Chinook salmon (*Oncorhynchus tshawytscha*) in the Central Valley" (FWS Cooperative Agreement / DCN #113322J006).

Tricia,

Below is a summary of the current status of the fall-run Chinook salmon sex-reversal project for the second quarter of its second year. If you have any questions, or require me to expand upon some point, please feel free to contact me.

Thank you,

Kevin S. Williamson
Ph.D. Candidate
Genomic Variation Lab
2403 Meyer Hall,
Department of Animal Science,
University of California,
One Shields Ave.
Davis, CA 95616

kswilliamson@ucdavis.edu
Lab: 530-752-6351
FAX: 530-752-0175

Status of controlled crosses from 2003

In the first quarter report we had mentioned that thirteen families involving XY female Chinook and 15 families involving genetically normal females had been produced. The offspring from 20 of these families have been transferred to separate rearing/holding tanks until they reach a large enough size to be dissected for observation of gross gonad morphology as a means to identify their phenotypic sex. Families of fish that either did not have high enough survival so that a statistically relevant analysis of offspring sex ratios could be made, or families from genetically normal female fish that had been produced in excess of planned experimental needs were culled before transfer to the rearing/holding tanks. In all, eight families involving XY female Chinook and 12 families involving genetically normal females were transferred to separate tanks.

Mortalities that occurred within each family were collected daily and stored in 100% ethanol until used for genetic analysis. The mortalities included very early stage embryos (~ 3-4mm long), eyed eggs, alevins, and free-swimming parr (fork length 45-60mm). Incidental mortalities will continue to be collected daily until the offspring are large enough to be used for dissection. Although the gonad morphology of these specimens cannot be ascertained due to the fact that the gonads have not yet developed to a point where they can be visually differentiated between the sexes, these specimens were genotyped using both the OtY1 and Growth Hormone pseudogene Y-chromosome markers. These offspring will be included in the analysis of offspring sex ratios for the family examined.

Discussion of preliminary genetic analysis of controlled crosses from 2003

Dead specimens collected from those families that were transferred to the holding/rearing tanks as well as those culled before transfer to the tanks have been genotyped at both Y-chromosome markers. Details regarding the methodology of genotyping mortalities of each family follows that given in section B, "Genetic screening to detect apparent sex-reversed male (XY female) fish" in the hatchery fish screening protocol "Evaluation of the inheritance of two sex-specific genetic markers in fall-run Chinook salmon from the Coleman National Fish Hatchery (USFWS) and Merced River Fish Hatchery (CDFG)" provided in the first quarter report for this project.

Genotyping data was examined for consistency of sex marker scores and was statistically tested, where possible, to evaluate the offspring sex ratio in each family. The consistency of sex marker scores was evaluated by merely observing whether or not the genetic markers corroborated one another in each individual tested. Statistical analysis, using a Chi-squared goodness of fit test, was used to evaluate whether or not the offspring sex ratio in each family deviated from the expected ratio of one female to one male.

A total of 624 offspring from the 2003 breeding experiments have been genotyped, so far, using both OtY1 and Growth Hormone pseudogene Y-chromosome markers. There has been no instance in which the genetic markers did not corroborate one another in a single individual. This is the same result as that obtained in our earlier

analyses (Williamson and May 2002 and 2003) of Fall-Run Chinook salmon using these genetic markers.

In only a few families have enough data been collected so that a statistically relevant analysis may be performed. Table 1 summarizes the observations and analysis of offspring genetic sex ratios that is currently available for the 2003 Fall-Run Chinook controlled breeding experiments. Offspring genetic sex ratios from four genetically normal females, and three XY-females are shown in Table 1. No significant deviation from a one male to one female offspring sex ratio was observed in the families originating from the genetically normal females. The average genetic sex ratio for offspring from the three XY-females was 3:1, males to females. Of the three families originating from the XY-females, two of the families had progeny genetic sex ratios that deviated significantly from a 1:1, male to female, ratio (Table 1). Family 93xB shows a three male to one female offspring genetic sex ratio. However, only 16 offspring from this family have been genotyped so far. Because of the limited number of offspring analyzed it is very likely that the statistical analysis of offspring sex ratio for family 93xB suffers from a lack of statistical power. In other words, due to limited sampling in family 93xB there is a low probability that the test will correctly reject the null hypothesis (in this case, 1 male to 1 female genetic sex ratio) when it is false. There appears to be a skewed genetic sex ratio in the offspring from sex-reversed males (XY females) that is not observed in the offspring from genetically normal females.

Families 87xB and 87xD, originating from XY female #87, suffered very high early mortality within 72 hours of fertilization. All of the individuals analyzed from these two families were either dead embryos or alevins that had lasted long enough to develop to a point where enough tissue was available to perform genotyping. The 16 offspring analyzed from family 93xB were either dead embryos or alevins as well. High mortality prior to hatch was also observed for families from genetically normal females collected the same day as female #87. Mike Kozart (Mgr., Merced River Fish Hatchery, CDFG) observed high mortality of eggs at the Merced River Fish Hatchery that were collected on the same date as phenotypic female fish #87. It is possible that the elevated mortality was due to the spike in water temperature that had occurred just prior and during the collection date for this fish (Mike Kozart, personal communication). The water source of the Merced R. Fish Hatchery is the Merced River.

Continuation of genetic analyses of controlled crosses from 2002

The evaluation of the controlled breeding experiments in 2002 was cut short due to a water quality accident at the UC Davis fish rearing facility 130 days after fertilization of the eggs (Williamson and May 2003). This prevented the analysis of most of the families created, and those that were analyzed suffered from a lack of statistical power to ascertain the genetic sex ratio of offspring due to limited sampling. Additional samples, dead embryos and alevins, from two families originating from an apparent XY-female (#174) used in the 2002 breeding experiments have been genotyped using both OtY1 and Growth Hormone pseudogene Y-chromosome markers. This additional data for the two families has been combined with that obtained last year. Table 2 summarizes

the observations and analysis of offspring genetic sex ratios for these two families as well as a genetically normal female (#88) Fall-Run Chinook in 2002. The average genetic sex ratio for offspring from this XY-female was 2.9:1, males to females. The genetic sex ratio for the offspring from the genetically normal female was 1.2:1, males to females. Both families (174xB, 174xC) from the apparent XY-female have an offspring genetic sex ratio that deviates significantly from a 1:1, male to female, ratio (Table 2). All available samples from 2002 have been genotyped. Again, there appears to be a skewed genetic sex ratio in the offspring from sex-reversed males (XY females) that is not observed in the offspring from genetically normal females.

Future plans for third quarter work

Genotyping and dissection of offspring from both normal and sex-reversed families will continue.

A poster summarizing the current results of the controlled breeding experiments will be presented at the Toxic Substances Research and Teaching Program Annual Symposium in San Diego, CA, April 24-25, 2004. If you so desire, I will provide you a copy of the poster.

A presentation concerning the results of this project will be made to the attendees of the 2004 Coast-wide Salmonid Genetics Conference to be held at the Hatfield Marine Science Center, Newport, Oregon, June 16-18, 2004.

References Cited

- Williamson, Kevin S., Bernie May. 2002. Incidence of phenotypic female Chinook salmon (*Oncorhynchus tshawytscha*) positive for the male Y-chromosome specific marker OtY1 in the Central Valley, California, U.S.A. *Journal of Aquatic Animal Health* 14(3): 176-183.
- Williamson, Kevin and B. May. 2003. Contaminant-induced sex-reversal of Fall-Run Chinook salmon (*Oncorhynchus tshawytscha*) in the Central Valley. Annual Report to U.S. Fish and Wildlife Service. September 2003. 12 pp.

Table 1: Chi-Squared Goodness of Fit analysis of genotype data, using the OtY1 and Growth Hormone pseudogene Y-chromosome markers, from the offspring of the 2003 Fall-Run Chinook controlled breeding experiments. The phenotypic female parent in each cross is either a genetically normal female or a sex-reversed male (SRM). The total number (N) of offspring genotyped in each family includes individuals that had died before development had proceeded to a point where sexual phenotype could be ascertained. An asterisk designates families that have a limited number of individuals available. As a result, statistical analysis of those families suffers from low power (the probability of rejecting a false null hypothesis).

Family ID	Phenotypic Female Parent	N	Expected Counts		Observed Counts		Observed M : F ratio	Chi-squared Value	p-value
			Males	Females	Males	Females			
84 x B	normal	132	66	66	64	68	1 : 1.1	0.068	0.90>p>0.75
85 X B	normal	82	41	41	46	36	1.3 : 1	0.988	0.50>p>0.25
85 x D	normal	66	33	33	36	30	1.2 : 1	0.379	0.75>p>0.50
105 x B	normal	102	51	51	41	61	1 : 1.5	3.539	0.10>p>0.05
87 x B	SRM	40	20	20	31	9	3.4 : 1	11.025	p<0.001
87 x D	SRM	32	16	16	23	9	2.6 : 1	5.281	0.05>p>0.025
93 x B*	SRM	16	8	8	12	4	3.0 : 1	3.062	0.10>p>0.05

Table 2: Chi-Squared Goodness of Fit analysis of genotype data, using the OtY1 and Growth Hormone pseudogene Y-chromosome markers, from the offspring of the 2002 Fall-Run Chinook controlled breeding experiments. The phenotypic female parent in each cross is either a genetically normal female or a sex-reversed male (SRM). The total number (N) of offspring genotyped in each family

Family ID	Phenotypic Female Parent	N	Expected Counts		Observed Counts		Observed	Chi-	p-value
			Males	Females	Males	Females	M:F ratio	squared Value	
88xB	Normal	48	24	24	26	22	1.2 : 1	0.188	0.75 > p > 0.50
174xB	SRM	56	28	28	43	13	3.3 : 1	15.018	p < 0.001
174xC	SRM	41	20.5	20.5	29	12	2.4 : 1	6.244	0.025 > p > 0.010

includes individuals that had died before development had proceeded to a point where sexual phenotype could be ascertained.