

Limited trawling is expected to resume in October. Since delta smelt will likely be a concern in the long term, moving the Chipps Island trawling site will be evaluated.

As a pilot study for 1996, kodiak trawling at Mossdale began September 4 on a 3 day/week basis to determine if any San Joaquin basin yearling chinook are coming into the delta in the fall and winter months. The effort is planned through March 15, when DFG Region 4 will resume monitoring for the annual index. No chinook had been captured through the end of September.

Contra Costa Canal Intake Entrainment

Jerry Morinaka

We used a sieve-net to sample fish entrainment every fourth day at the Contra Costa Canal (on the discharge side of Pumping Plant 1) during July, then switched to once a week in August. The predominant fish species captured in July and August was juvenile American shad (mean fork length = 25). Chinook salmon, delta smelt, longfin smelt, and splittail were not captured in the sieve-net during any of the sampling efforts. Although August marked the end of the initial 3-year sampling program, sieve-net sampling will resume in November and continue until the diversion is screened (projected to be October 1998).

In July, we monitored in the intake channel of the Mallard Slough Pumping Plant and outside the channel in Suisun Bay, using tows with an egg and larval net and a modified tow-net. The egg and larval net sampling captured delta smelt in Suisun Bay (0.0387 smelt/m³), but delta smelt were not captured in the intake channel. Longfin smelt were captured in Suisun Bay with both the egg and larval net (0.0097 smelt/m³)

and the modified tow-net (0.0104 smelt/m³), but not in the intake channel. Salmon and splittail were not captured in either gear type. Monitoring was discontinued after July because the diversion was no longer being used by Contra Costa Water District.

Sport Fish Project

Dave Kohlhorst

We completed two of three scheduled monthly juvenile sturgeon surveys between Sherman Island and San Pablo Bay. This survey, using baited set-lines, is designed to sample 40-116cm sturgeon with the aim of estimating year-class strength. A fin ray, used to age sturgeon, is removed from a subsample of the catch for age determination and development of an age-length key. The length-frequency distribution is distinctly multimodal, with an obvious group of fish at 44-52cm, a wider mode at 66-82cm, and a large number mostly > 100 cm. No 54-64cm sturgeon were caught. Although no fin rays have been sectioned for aging, past age-length data suggest that the smallest mode represents the 1995 year class. The second, intermediate-sized group probably consists of year classes from the early 1990s, and broad grouping > 100 cm encompasses year classes from the 1980s, including cohorts from the strong 1982 and 1983 year classes.

Fish Treadmill Studies

Darryl Hayes

The long-awaited operation of the large fish stamina testing device is finally a reality. Initial hydraulic tests indicate the device is able to operate within the expected hydraulic criteria. The 1:2.5 scale model was well worth the effort because of design insights and modifications during the study that led to the prototype design. The complex free and

forced vortex actions in the circular flumed facility can be well controlled and easily replicated for the proposed experiments. The laboratory is being equipped with the necessary water quality control features and fish holding facilities.

A meeting with agency technical representatives is expected to result in testing priorities; testing protocols; and an outline of responsibilities for the first year of testing, scheduled to begin this fall or as soon as the holding facilities are completed.

CALFED Fish Facilities Development

Darryl Hayes

During the Phase II process of the CALFED Bay/Delta Program, fish facilities planning will be an important consideration in defining components of the preferred alternative. A focused project work team, jointly led by DFG and DWR, will study feasibility, operations, and fishery issues associated with the remaining alternatives. Conceptualized facilities, fish screen criteria, and protection goals will be established and included in the proposed program EIR/EIS.

New information on fish facilities development will be limited within the next year, so Phase II alternatives and criteria will be based on past efforts, existing criteria, and judgment of fish facility experts. Limited information will be available on fish pump feasibility from the Red Bluff Research Pumping Plant studies and on appropriate velocity criteria for delta fish species from the "fish treadmill" studies. System simulation and hydraulic modeling studies of alternatives and establishment of pre-project fish monitoring will also be emphasized. Agency and consulting experts will assist in the new project work team's effort on selection of proposed facility options.

The Question of Run Identity for the Winter Run Take in 1996: Some Genetic Perspectives

Michael A. Banks, Marco J. Calavetta, Sheila Greene, Vanessa Rashbrook, and Dennis Hedgecock

Correctly identifying the origins of juvenile chinook salmon taken at the state and federal water projects is necessary to comply with legal protections afforded threatened or endangered stocks. For example, water projects must consult with the National Marine Fisheries Service if take of winter run at the salvage operations has reached 1% of the estimated outmigrating population of smolts (NMFS 1993, 1995), with the limit being 2%. (If the take reaches the 2% level, the agencies reinstate consultation to determine appropriate action.) This 2% "red light" condition was reached in April 1996 (Anon. 1996). Researchers and managers also need positive run identification techniques to help determine which stocks are being harvested, to determine timing of outmigration, and even to identify the run of salmon taken by poachers. Traditionally, the length distribution of juvenile fish, given the time and locality where captured, has been used to determine run identity (Fisher 1992). Today, however, methods of molecular biology and population genetics promise to resolve run identity with much greater accuracy and precision.

This article provides a brief overview of these methods and an example of their application to estimate the composition of two samples of juvenile chinook in the winter-run length. The first sample of juveniles (RBDD'95) was collected by rotary screw trap at the Red Bluff Diversion Dam between August 4 and December 5, 1995; all juveniles were within the winter-run length criteria. The second sample (SWP'96) was collected at the SWP facility between January 9 and April 3, 1996;

this sample comprises 17% spring run, 14% late-fall run, and 69% winter run according to length criteria.

DNA analyses used to identify the races of fish in these samples consist of:

- A genetic profiling of baseline populations of known run identity, *ie*, collections of either spawning adults, post-spawn carcasses, or young juveniles collected on spawning grounds.
- A statistical test to determine if each of the two samples of juveniles consists of single populations or are mixtures from more than one run.
- An allocation of juvenile samples to their likely run origins.

These analyses are preliminary. They are included to illustrate the process and demonstrate its potential.

Molecular Techniques for Discrimination of Chinook Salmon Runs

Protein variation has been used since the early 1970s to understand the genetic affinity of Pacific salmon species and populations. More recently, variation in the DNA sequence of the mitochondrial genome (mtDNA) has been applied to the task. Variation among chinook salmon stocks of the Central Valley for either of these classes of molecules is insufficient to accomplish the run discrimination needed in the delta and elsewhere. Therefore, we have isolated and characterized some nuclear DNA markers, known as microsatellites, that undergo evolutionary structural changes three to five times as fast as the rate of change for proteins or mtDNA (Edwards *et al* 1991; Hearne *et al* 1992; Weissenbach *et al* 1992; Queller *et al* 1993). This class of DNA markers ought, therefore,

to reveal differences among the recently evolved and closely related chinook runs of the Central Valley. Of 41 available microsatellites isolated from Pacific salmon, 5 show strong potential for run discrimination in the Central Valley river system. The first such diagnostic marker to be isolated, a microsatellite we have named *Ots-2*, is transmitted from generation to generation according to the laws of inheritance first discovered by Gregor Mendel in his pea studies (Box A; Banks *et al* 1996). Population data for this microsatellite are now extensive enough to provide an example of run determination and mixed stock analysis. Current research is actively accumulating data for the other four microsatellite loci that show potential for increasing the power for run discrimination and mixed stock analysis.

We now step through a method for analysis of data derived using these techniques. The RBDD'95 and SWP'96 juvenile chinook samples will be the working examples of unknown populations that we will test for mixture and for which we will perform mixed stock analysis.

Step 1 Genetic Profiling of Baseline Populations

Representative populations of the four major runs of chinook salmon spawning in the upper Sacramento River basin have been characterized for *Ots-2* variation (Table 1). Although we have examined all winter-run brood stock collected for the captive propagation and hatchery supplementation programs, the winter run is represented here by a sample of post-spawn carcasses collected on the Sacramento river near Redding from June

through August 1995. The spring run is represented by samples from Mill Creek in 1995 and Deer Creek in 1993 and 1994. Pooled with these spring-run samples is a collection of fish from the upper Sacramento River

that are genetically similar or indistinguishable from the Mill and Deer creeks spring run using *Ots-2* and other markers. The fall run is represented by a 1995 collection from the upper Sacramento River, and the

late-fall run is represented by a 1995 sample of fish returning to Coleman National Fish Hatchery on Battle Creek.

Box A Inheritance of Microsatellite DNA Markers.

What is a microsatellite? A microsatellite is a simple tandem repeat of the nucleotide base-pairs that make up the double-stranded ladder of the DNA molecule. In the case of *Ots-2*, the dinucleotide base-pair AC/TG is repeated, commonly from 17 to 27 times at one specific location in the chinook salmon genome. This variably repeated portion of the genome can be specifically amplified by the polymerase chain reaction or PCR (of O.J. Simpson trial fame), using synthetic pieces of DNA called "primers" that match the unique, 16 base-pair sequences that immediately flank both sides of the *Ots-2* repeat element. PCR permits amplification of *Ots-2* from the tiny amount of DNA that can be extracted from 1mm² fin clip samples. In the winter run, the most common *Ots-2* sequence is a 17-repeat of the AC//TG core, which upon PCR amplification yields a large quantity of DNA that is 66 base pairs in length (ie, 66 = (17 repeats of the two base-pair, AC//TG core element) + (16 base-pairs in each of 2 flanking primers)). The next most common *Ots-2* sequence in the winter run is a 19-repeat, which yields a PCR product of 70 base-pairs. These two forms of *Ots-2*, which we call L and K, are readily distinguished by electrophoretic separation of the PCR product on polyacrylamide gels that sieve DNA molecules on the basis of size. Thus, the smaller L form migrates faster and farther than the larger K form.

Ots-2 is inherited in the winter run according to Mendel's laws. Each chinook salmon carries two copies of the *Ots-2* DNA marker that it inherited, one from its father and one from its mother. Both copies are amplified in a PCR reaction from a fin-clip sample. Examination of winter-run captive broodstock and their parents allows the inheritance of the *Ots-2* marker to be visualized and confirmed. A male with an LK phenotype was mated to a female with an LL phenotype and produced the ten offspring shown here. The ratio of 1:1, LL:LK phenotypes in the progeny agrees with Mendel's law of random segregation of alleles into gametes and assortment into two genotypes in the offspring.

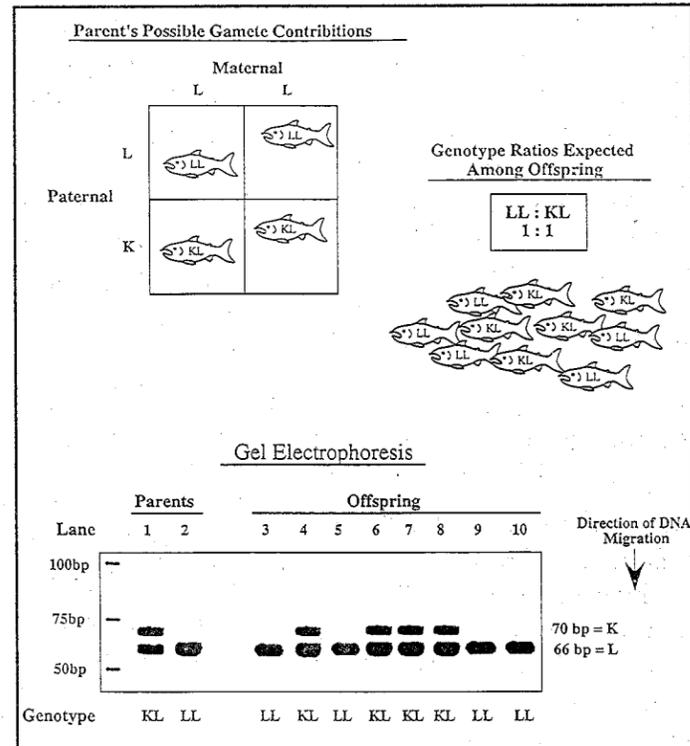


Table 1
CHINOOK SALMON POPULATIONS UNDER STUDY

Sample Size	Dates Sampled	Life Stage	Baseline Location	Provided by*	Grouping	
Winter-Run Carcasses	88	06/05/95 - 08/12/95	Adult	Redding	1	Winter
Deer Creek Spring	37	04/25/94 - 05/24/94	Juveniles(1A)	Deer Creek	2	Spring
Mill Creek Spring	3	09/24/93 - 10/07/93	Adult	Deer Creek	2	Spring
Brood Year 95 Spring	14	10/04/95 - 10/30/95	Juveniles	Mill Creek	2	Spring
Upper Sacramento River Fall	38	06/20/95 - 08/14/95	Adults	Redding	4	Spring
Coleman Hatchery Late-fall	79	11/14/95 - 11/27/95	Adults	Redding	3	Fall
Red Bluff Diversion Dam 95	79	01/03/95 - 01/24/95	Adults	Anderson	3	Late-fall
State Water Project 96	91	08/04/95 - 12/05/95	Juveniles	Red Bluff	1	Unknown
	174	01/09/96 - 04/03/96	Juveniles	Byron	5	Unknown

* 1 = U.S. Fish and Wildlife Service, Fishery Resource Office
2 = Department of Fish and Game, Frank Fisher
3 = U.S. Fish and Wildlife Service, Coleman National Fish Hatchery
4 = Department of Fish and Game, Inland Fisheries Division
5 = Department of Water Resources, Environmental Services Office

One particular form, or allele, of the *Ots-2* microsatellite, which has been assigned the alphabetic designation "L", occurs in high frequency (70-80%) in all the winter-run populations sampled since 1991. This L allele, however, only occurs in low frequency (<5%) in all other chinook populations investigated so far. Moreover, when we consider genotypes of individuals (which inherit one allele of the *Ots-2* marker from their father and one allele from their mother), we find that LL homozygotes comprise more than 60% of the winter-run population but have not been seen in samples from any of the other runs. We have now characterized *Ots-2* from more than 1,000 chinook salmon from the Central Valley, and the LL *Ots-2* homozygote remains remarkably diagnostic for the winter-run population (Figure 1).

Step 2 Genotypic Proportions in Mixed Samples

Juvenile chinook captured at Red Bluff Diversion Dam in late 1995 have a high proportion of the LL homozygous genotypes and, thus, resemble the winter-run carcass sample (Figures 1 and 2). In contrast, smolts salvaged at Skinner Fish Facility (SWP'96) in 1996 have a low frequency of the LL genotype, which suggests strongly that these juveniles did not originate primarily from the winter-run population (Figures 1 and 3). Of the 174 fish in the SWP sample, however, 6 are LL genotypes and are likely of winter-run origin. The presence of a few winter-run fish in a largely non-winter-run sample suggests that this group of smolts is a mixture originating from different spawning populations.

The relative proportions of genotypes in each of these two juvenile samples can be examined for evidence of population admixture. The most

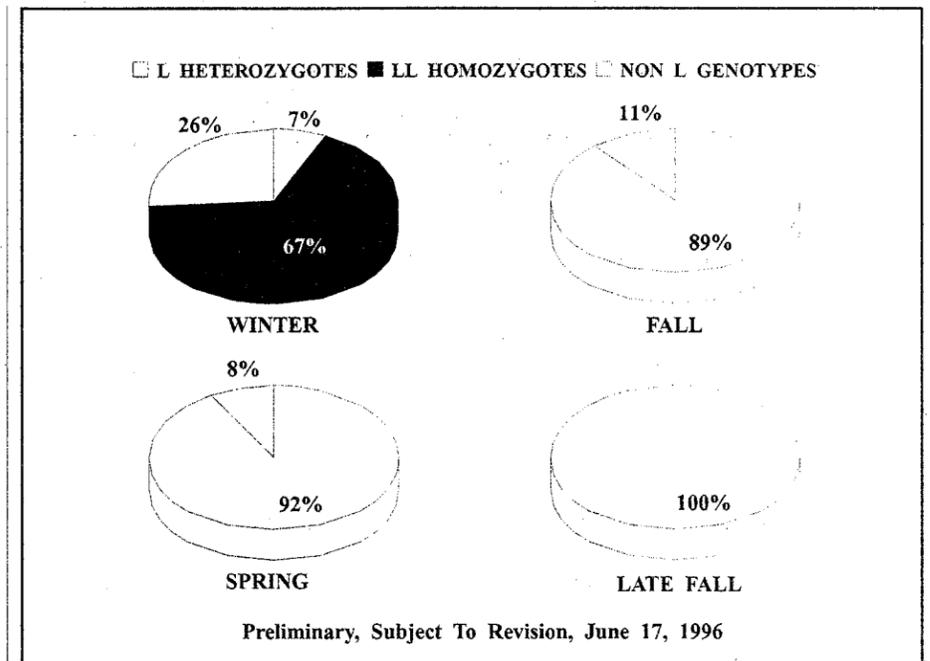


Figure 1
OTS2 GENOTYPE FREQUENCIES IN 1995 WINTER-RUN CARCASSES AND FALL-RUN, SPRING-RUN, LATE-FALL-RUN CHINOOK SALMON

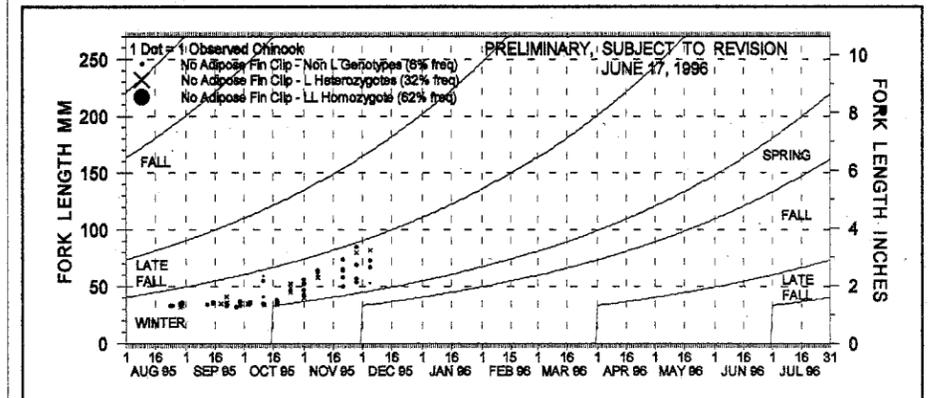


Figure 2
CHINOOK SALMON OBSERVED IN THE ROTARY SCREW TRAP AT RED BLUFF DIVERSION DAM, 8/4/95 - 12/5/95

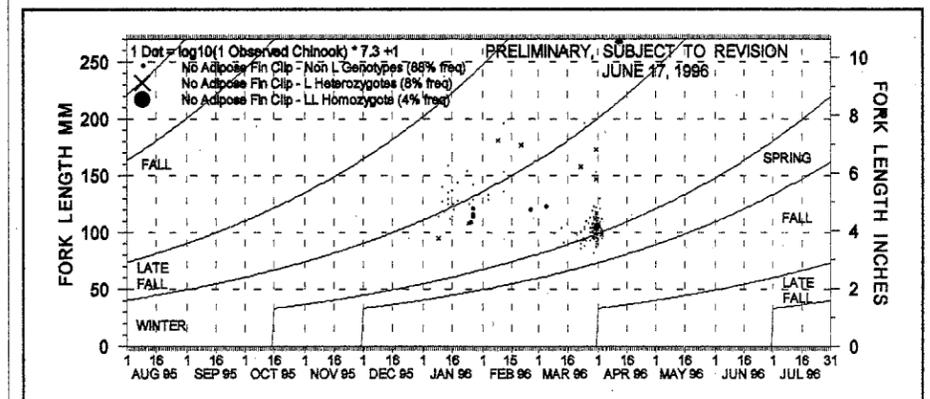


Figure 3
CHINOOK SALMON SALVAGED AT SWP DELTA FISH FACILITIES. 10/1/95 - 5/31/96

Box B Hardy-Weinberg Equilibrium Proportions of Genotypes in Randomly Mating Populations

The Hardy-Weinberg principle provides a useful measure for determining whether a sample of juvenile fish comes from one or more runs. The principle results from the simple fact that genes (microsatellite alleles in our case) assort into gametes and combine at random in a single population or run. Consider alleles L and K with frequencies p and q in the sperm and eggs of a typical winter-run population. If these gametes unite at random, the resulting juveniles will be of three genotypes LL, LK or KK with frequencies p^2 , $2pq$ and q^2 respectively (see Punnett square).

The Hardy-Weinberg principle now assures us that in the absence of migration, mutation, selection and genetic drift, the proportions of LL, LK, and KK genotypes, $P (= p^2)$, $Q (= 2pq)$ and $R (= q^2)$ respectively, will remain constant from generation to generation. A demonstration follows.

First the frequency of gametes carrying the L allele is: the sum of P times two (since there are two L alleles in the genotype LL) and Q (since there is one L allele the genotype LK) divided by two (since each genotype has two alleles).

$$p = (2P + Q)/2 = P + Q/2 \quad 1$$

Likewise, for the K allele:

$$q = (2R + Q)/2 = R + Q/2 \quad 2$$

Now consider all possible matings among the three genotypes and their frequency of occurrence in a randomly mating population.

Matings	Frequency of Mating	Offspring Genotype Frequencies		
		LL	LK	KK
LL x LL	p^2	1	0	0
LL x LK	$2PQ$	$1/2$	$1/2$	0
LL x KK	$2PR$	0	1	0
LK x LK	Q^2	$1/4$	$1/2$	$1/4$
LK x KK	$2PR$	0	$1/2$	$1/2$
KK x KK	R^2	0	0	1
Totals for Next Generation		P'	Q'	R'

Where: $P' = P^2 + 2PQ/2 + Q^2/4 = (P + Q/2)^2 = p^2$ (from above 1) = P
 $Q' = 2PQ/2 + 2PR + Q^2/2 + 2PR/2 = 2(P + Q/2)(R + Q/2) = 2pq$ (from above 1+2) = Q
 $R' = Q^2/4 + 2PR/2 + R^2 = (R + Q/2)^2 = q^2$ (from above 2) = R

Thus the frequencies remain constant from generation to generation, the essential feature of the Hardy-Weinberg principle.

If frequencies among samples of juveniles deviate significantly from this expectation then the sample likely contains juveniles from more than one run. All populations under study were tested for Hardy-Weinberg allele frequencies using a computer program developed by Zaykin and Pudovkin (1993).

Now let us consider an example of failure because of mixture:

Typical Winter-Run Population	Other Run
$p = 0.8$ $q = 0.2$	$p = 0.05$ (frequency of L allele) $q = 0.95$ (frequency of K allele)

Genotype frequencies:

LL	LK	KK	LL	LK	KK
0.64	0.32	0.04	0.0025	0.095	0.9025

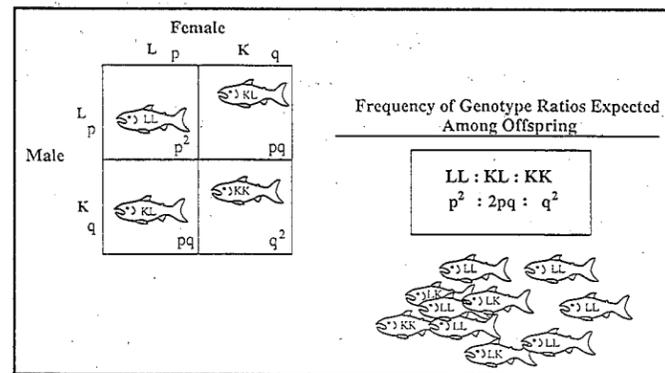
Now assume a 100:100 mixture of these two runs:

Genotype frequencies:

Observed	LL	LK	KK
	64.25	41.5	94.25
$p = 2(64.25) + 41.5 = 170/400 = 0.425$			
$q = 2(94.25) + 41.5 = 230/400 = 0.575$			
(400 is the total number of alleles since there are 200 individuals in the mixture, each with two alleles)			

Thus expected frequencies assuming Hardy-Weinberg:

LL	LK	KK
36.125	97.75	66.125
$\chi^2 = (64.25 - 36.125)^2/36.125 + (41.5 - 97.75)^2/97.75 + (94.25 - 66.125)^2/66.125 = 66.228$		
This has a probability of 0.0000000000000416!!!!		



Thus, the mixed sample fails the Hardy-Weinberg principle. It also fails because of deficiencies of heterozygotes, and an excess of homozygotes, which is called the "Wahlund effect" after its discoverer (Wahlund 1928). The excess of LL homozygotes noted above as evidence for mixture in the SWP sample is classic Wahlund effect.

fundamental principle of population genetics, the Hardy-Weinberg principle, holds that random mating between males and females produces, in the next generation, offspring genotypes whose relative proportions are described by the binomial distribution (Box B). Indeed, previous studies of protein variation have shown that the Hardy-Weinberg principle applies well to Pacific salmon populations, and statistical tests show that the relative proportions of *Ots-2* genotypes in all of our baseline populations conform to those expected under the Hardy-Weinberg principle. Tests of the RBDD'95 and SWP'96 samples reveal that although the former sample passes, the latter sample fails, providing statistical support for the notion that the SWP'96 sample is indeed a mixture of offspring from more than one spawning population.

Step 3 Mixed Stock Analysis

Mixed stock analysis, which was perfected for management of salmon ocean harvest, is a statistical procedure that estimates the most likely composition of a mixed sample using genetic information on the mixed sample and on the spawning populations that could have contributed to it (Utter and Ryman 1993). In essence, MSA is a maximum likelihood computer algorithm that finds the fraction of each baseline population (both known and unknown) required to yield the genotypic proportions observed in a mixed sample, in this example, the RBDD'95 and SWP'96 juvenile samples. The *Ots-2* data for the four major spawning runs and for these juvenile samples were input as baseline populations and mixed samples, respectively, into such a computer

program (Statistical Package for the Analysis of Mixtures, or SPAM) obtained from the Alaska Department of Fish and Game genetics laboratory. The MSA for the RBDD'95 sample assigns 84.9% to winter run, 15.1% to spring run, and 0% to either of the other two runs. The MSA for the SWP'96 sample allocates 35.4% to spring run, 31.3% to fall run, 27.5% to late-fall run, 5.2% to winter run, and 0.6% to other "unknown" populations (Figure 4).

Conclusions

From the *Ots-2* data presented in our mixed stock analysis example, we conclude:

- As expected on the basis of time and place of capture, the Red Bluff fish are largely winter run, conform to Hardy-Weinberg genotypic proportions, but show a 15% spring run component in the MSA.
- Juvenile chinook salmon captured at the SWP in the first quarter of 1996

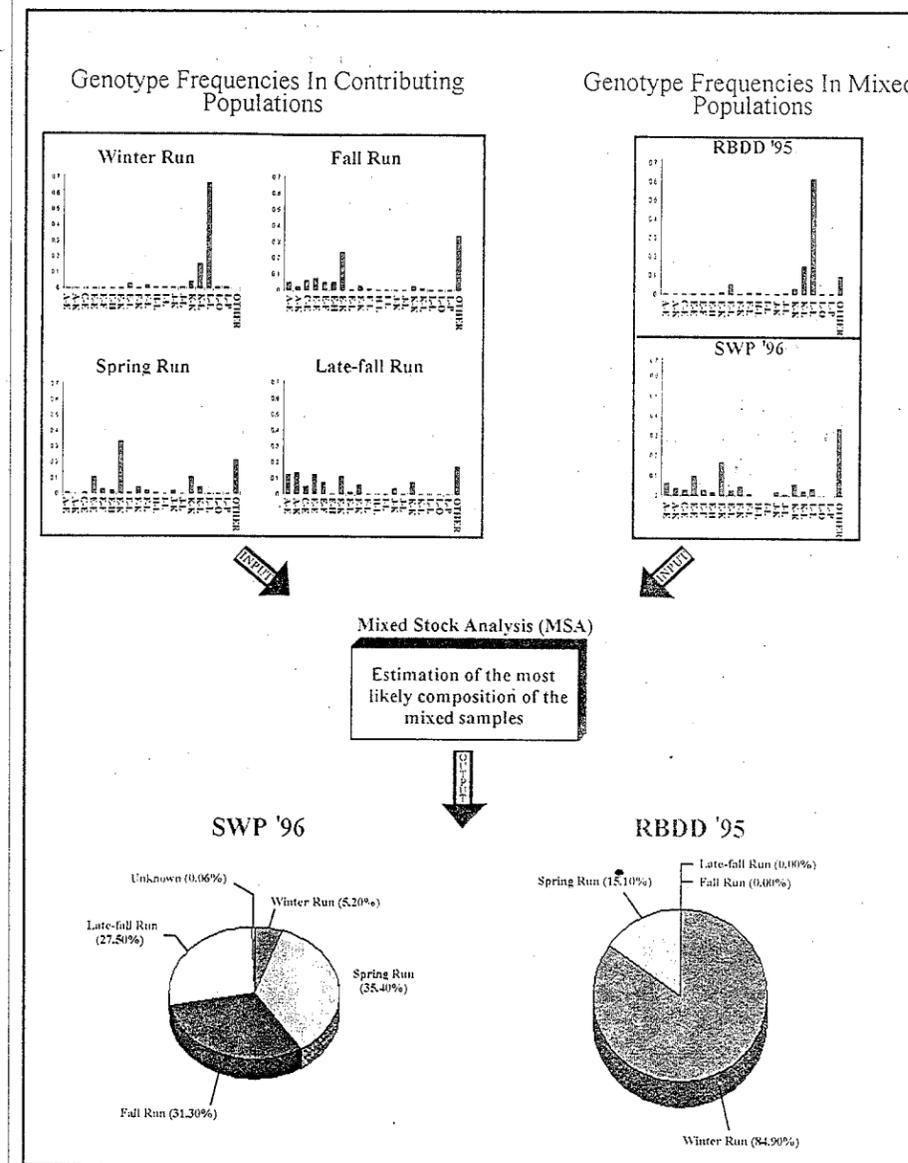


Figure 4
OUTLINE OF MIXED STOCK ANALYSIS PROCEDURE
Genotype frequency data for mixed populations (RBDD'95 & SWP'96) are compared with data from known contributing populations (W,S,F,& LF) to make estimation of the most probable composition of the mixed populations (pie charts).

are of mixed origin, as judged by the failure of the test for Hardy-Weinberg genotypic proportions in a randomly breeding population.

- On the basis of MSA, the SWP fish are a mixture of the major runs, with winter run comprising about 5% of fish in the sample provided.

We have presented an example of the type of molecular and population genetic analyses that now promise to resolve the run identity of chinook salmon captured at the water projects. Such an analysis may change substantially the conclusions of run identity

reached from applying traditional length criteria for identifying winter run. However, to accomplish this MSA with a high degree of statistical confidence and in "real time" (at least weekly), we need:

- Multiple genetic markers to increase the precision with which samples of the mixed take can be allocated to run.
- More and better data on baseline populations, *ie*, larger samples of all chinook spawning populations that potentially contribute to the take of juveniles in the delta.

- Validation of the MSA both by computer simulations and blind tests of mixed samples of known composition.

All these needs are being addressed by current research with the goal of having MSA operational in 1997. Improved methods for run diagnosis will provide a resource for valuable understanding of chinook salmon biology in general. Application of run diagnosis to samples from all stages of the life cycle will enhance perspectives for both freshwater and marine environs.

References

- Anon. 1996. Winter Run Take. Interagency Program *Newsletter* (8):2:12.
- Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1996. Research on chinook salmon stock structure using microsatellite DNA. *Bull. Natl. Res. Inst. Aquaculture*. Suppl. 2:5-9.
- Edwards, A., A. Civitello, H.A. Hammond, and C.T. Caskey. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am. J. Hum. Genet.* 49:746-756.
- Fisher, F. 1992. *Chinook Salmon, Oncorhynchus tshawytscha, Growth and Occurrence in the Sacramento-San Joaquin River System*. DFG, Inland Fisheries Division Office Report.
- Hearne, C.M., S. Ghosh, and J.A. Todd. 1992. Microsatellites for linkage analysis of genetic traits. *Trends Genet.* 8:288-294.
- National Marine Fisheries Service. 1993. *Long-Term Operation of the Federal Central Valley Project and the California State Water Project*. Endangered Species Act Section 7 Consultation, Biological Opinion. National Marine Fisheries Service, Southwest Region, Long Beach, CA. 81 pp.
- National Marine Fisheries Service. 1995. *Amendment to the Long-Term Operation of the Federal Central Valley Project and the California State Water Project based on the Principles of Agreement on Bay-Delta Standards between the State of California and the Federal Government*. National Marine Fisheries Service, Southwest Region, Long Beach, CA. 13 pp.
- Queller, D.C., J.E. Strassmann, and C.R. Hughes. 1993. Microsatellites and kinship. *Trends Ecol. Evol.* 8:285-288 + centerpage.
- Tautz, D., and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nuc. Acids Res.* 12:4127-4138.
- Weissenbach, J., G. Gyapay, C. Dib, A. Vignal, J. Morissette, P. Millasseau, G. Vaysseix, and M. Lathrop. 1992. A 2nd-generation linkage map of the human genome. *Nature* 359:794-801.
- Utter, F., and N. Ryman. 1993. Genetic markers and fisheries. *Fisheries* 18:11-21.
- Wahlund, S. 1928. The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity. (German) *Hereditas* 11:65-106.
- Zaykin, D.V., and A.I. Pudovkin. 1993. Two programs to estimate significance of chi-square values using pseudo-probability tests. *J. Hered.* 84:152.

Results of 1996 Coded-Wire Tag Smolt Survival Experiment in the San Joaquin River Delta

Pat Brandes

Adult salmon escapement in San Joaquin tributaries has fluctuated dramatically for many years, but since 1989, total annual basin escapement has not exceeded 5,000 fish. There are statistical relationships between escapement and production and flow and CVP/SWP exports 2½ years earlier (DFG 1987; USFWS 1991). As flows increase and exports decrease, adult escapement and production increases. In an attempt to identify management actions that could increase smolt survival (a likely variable responsible for the adult relationships), several smolt survival studies have been conducted. In general, results of survival experiments in the southern delta indicate that:

- Survival to Chipps Island for coded-wire tagged smolts has become increasingly poor over the past 7 years, and recovery of the tagged smolts at the fish facilities has decreased markedly.
- Survival through the San Joaquin side is much lower than in the Sacramento side of the delta.
- Survival down the mainstem San Joaquin River is generally about twofold greater than for smolts emigrating down upper Old River, suggesting that a full barrier at the head of Old River could increase smolt survival and, thus, benefit the adult population.

Survival experiments in 1992 and 1994 focused on evaluating a temporary barrier at the head of Old River as a means to increase smolt survival. In 1992, water temperature increased after barrier installation, preventing unbiased evaluation, and in 1994, all survival indices were too low to differentiate a benefit associated with the barrier. In 1993 and 1995, high

flows prevented installation of the barrier.

Based on experience, the 1996 study design included a barrier at the head of Old River for the entire experimental period to get replication with a barrier and determine if survival was higher than in past years without a barrier. However, barrier installation was delayed due to permitting issues and was removed early due to flooding concerns. The barrier was closed fully on May 11 and was partially breached on May 16.

We also increased release numbers in 1996, from 50,000 to 100,000 for the first two sets of releases at Mossdale and Dos Reis, in an attempt to improve precision via greater recoveries at Chipps Island. Because of the limited number of marked fish available, the later releases (May 9 and 16) at Dos Reis were restricted to groups of 50,000. Due to the daily uncertainty of the barrier status in 1996, some changes in release sites and release numbers were necessary, deviating from the study plan.

Smolt survival during 1996 was evaluated under a San Joaquin flow of about 6,000-12,000 cfs and total CVP/SWP exports of about 1,500 cfs during the April 15-May 15 "pulse flow" period, resulting in a 4:1 inflow/export ratio. Exports increased to about 3,000 cfs on May 16 and increased again to 8,300 cfs on May 21. By May 28, combined exports had resumed to pre-pulse-flow levels of 10,300 cfs.

In addition to barrier evaluation, several other concerns with past experiments were addressed in 1996.

Most southern Delta survival experi-

ments have been conducted with smolts from Feather River Hatchery, raising the question of whether using out-of-basin smolts has biased results. The 1996 study was designed to determine if smolts from Merced River Fish Facility released at Dos Reis Park on the San Joaquin River survived to Jersey Point at a higher rate than smolts from Feather River Hatchery released at the same location. Survival to Jersey Point was estimated by comparing differential survival to Chipps Island for the Dos Reis and Jersey Point groups from each facility.

To assess differential temperature tolerance between stocks, a subset of about 200 fish from each paired group released at Dos Reis was held in submerged cages for 48 hours to determine immediate and differential mortality. An additional 33 (Merced) and 34 (Feather) fish were sacrificed for a variety of physiological tests (internal parasites, bacteria, organosomatic analyses, ATPase assay, triglyceride levels, and stress glucose response). Another 12 fish from each facility were used to assess osmoregulatory ability. Additional health monitoring was done at Chipps Island on May 6 to assess change in health status of emigrating smolts.

To identify a potential mechanism for low southern delta survival, the study assessed the percentage of smolts diverted from the mainstem San Joaquin River into Turner Cut. Data from this part of the study have not been completely processed, so results are not included in this article.

The main part of the 1996 study consisted of two sets of CWT releases. The first releases were at Mossdale on April 15, upstream of Turner Cut on April 17, and Jersey