

# FRED Reports

GENETIC MARKING AT TUTKA LAGOON HATCHERY  
AUGUST 1981  
by  
Carmen Olito  
and  
Rob Davis  
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**Alaska Department of Fish & Game**  
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## ABSTRACT

The frequency of an electrophoretically detectable variant of the enzyme  $\alpha$ -glycerophosphate dehydrogenase was increased from .087 to .108 in Tutka Lagoon Hatchery pink salmon in August 1981. The selection procedure necessary to achieve this was incorporated into the regular egg-take operation at Tutka with a minimal amount of interference. At least two more generations of selection are needed to attain an easily detectable level of this AGP variant. This selection must be more intense--screening at least twice as many males as in 1981. With a small crew working exclusively on the genetic marking project, this tag can easily be developed in the odd-year pink salmon at the Tutka Lagoon Hatchery.

## INTRODUCTION

Electrophoresis has been used extensively in the last ten years to describe protein variation in salmon. This variation is seen as banding patterns on gels, which are interpreted as genotypes. As the banding patterns are heritable and occur in many populations, they can be used as a genetic mark. Frequencies of the genes producing these patterns are different in each salmon stock. In most areas, though, the magnitude of difference is not sufficient to identify a stock with certainty.

One (or more) variable gene locus can be used for stock identification. After a locus has been chosen, artificial selection for the variant (or less common) gene in one population will magnify the frequency difference, uniquely marking this population. The major advantages in genetic marking are (1) the ability of the marks to perpetuate themselves, and (2) there are no obvious negative characteristics associated with them.

If hatchery fish are marked genetically, they will be the unique population in a system. The distinction will then enable us to examine the interactions of hatchery fish with wild populations. These include the evaluation of hatchery success (or contribution to the mixed fishery), juvenile migration, and straying.

In August 1981, the Genetics Laboratory initiated attempts to develop a genetic mark in the pink salmon population at Tutka Lagoon Hatchery. The objectives of this project are: (1) find a suitable biochemical genetic variant to be used as a genetic marker; (2) increase the frequency of this variant through artificial selection; (3) determine the feasibility of developing a genetic mark in a production facility, and (4) use the genetic marker to evaluate hatchery production.

Prior screening of Tutka pink salmon suggested the use of a variant form of the enzyme  $\alpha$ -glycerophosphate dehydrogenase (AGP) as a genetic marker. Breeding experiments done by Aspinwall (1973) confirm the genetic basis of the electrophoretically detected variation. The three AGP genotypes found are characteristic of a dimer encoded by one autosomal locus with two codominant genes, AGP(100) and AGP(200). The gel pattern for each genotype is unambiguous. Carriers of the variant gene AGP(200) have either one band with twice the mobility of the common gene (homozygote) or a total of 3 bands (heterozygote).

## METHODS

Tutka Lagoon adult pink salmon were separated by sex and stage of ripeness and held in saltwater pens until spawning. Ripe or near-ripe males were subjected to genetic screening the day before they were to be spawned. Each male was netted, tagged with Floy anchor tags, white muscle biopsied with a 6 mm dermal punch, and the fish returned to its pen. The biopsies were analyzed electrophoretically in a small laboratory set up in the hatchery. Standard electrophoretic and staining techniques were used in the lab (May 1975; Harris and Hopkinson 1977).

Tag numbers of any male carrying the variant AGP gene in one or two doses were noted. During the regular hatchery spawning operation the following day, these selected males were used to fertilize a portion of the eggs with a male to female ratio of 1:4. All unselected males were destroyed. The selected progeny were incubated separately but received no special treatment in the hatchery. The rest of the hatchery eggs were fertilized with non-screened males.

## RESULTS

During two weeks of spawning, 1,883 males were electrophoretically screened. Of these males, 297 were selected to spawn with 1,248 unselected females. Fecundity was 1,400 eggs per female which resulted in 1.8 million eggs subjected to the genetic marking. Assuming male and female initial AGP gene frequencies are similar, the variant gene frequency was altered from .087 to .318 in the selected progeny.

At the Tutka Lagoon facility, 20.3 million pink salmon eggs were taken in 1981. The 1.8 million genetically marked eggs comprise 8.9% of the hatchery population. This proportion changes the selected variant frequency from .087 to .108 in the whole Tutka population. This difference is not large enough to be detected using a reasonable sample size.

To date there have been no differences in egg performance between select and nonselect egg lots. Selected egg survival was 83.4% to the eyed stage. This is not significantly different from the overall hatchery survival of 80%.

## DISCUSSION

As a result of the artificial selection, the AGP (200) gene frequency in the Tutka Lagoon pink salmon population has been permanently increased. By repeating the selection procedure in the next two to three generations (1983 and 1985), the frequency difference will be increased and Tutka pink salmon genetically marked. This selection over a few generations is preferable. It is slower and more fish genomes can be incorporated into the select group, minimizing the potential loss in genetic variation.

Our initial goal in 1981 was to genetically mark 25% of the odd-year progeny. The number of males to be screened for this project was based on AGP frequencies found in 1980 Tutka pink salmon and the expected hatchery goal of 15 million eggs. The 1981 AGP(200) frequency was 4% lower than estimated, and the hatchery increased production from 15 to 20 million eggs. As there were fewer heterozygotes than we expected for the selection procedure only 8.9% of the 1981 progeny were marked. We did not screen females because it was important to minimize their handling.

Determining the feasibility of incorporating a genetic mark into the population at a production facility was one of the original objectives in this project. The low-keyed trial selection done in 1981 did not interfere very much with hatchery egg take operations. To attain a useable genetic mark, though, selection in the next few generations will have to be more intense. Tables 1A, 1B, and 2 describe three possible breeding

Table 1. Change in gene and genotype frequencies after selection.

A. Select 25% of the population

Returning Adults	Gene Frequencies		Genotype Frequencies			No. of Males to be Screened	No. of Selected Eggs
	100	200	100/100	100/200	200/200		
Year							
1983	.892	.108	.796	.187	.017	4,380	5,000,000
1985	.838	.162	.710	.255	.036	3,070	5,000,000
1987	.787	.213	.633	.307	.060	2,435	5,000,000
1989	.741	.259	.565	.350	.085	--	--

B. Select 50% of the population

Returning Adults	Gene Frequencies		Genotype Frequencies			No. of Males to be Screened	No. of Selected Eggs
	100	200	100/100	100/200	200/200		
Year							
1983	.892	.108	.796	.187	.017	8,760	10,000,000
1985	.783	.217	.626	.313	.062	4,764	10,000,000
1987	.692	.308	.493	.397	.110	3,522	10,000,000
1989	.617	.383	.394	.446	.160	--	--

- Assumptions:
1. Hatchery goal remains at 20 million eggs each year.
  2. Fecundity remains at 1400 eggs per female.

Manpower Needed: For each day 500 males are screened, a crew of 4 people will sample for 5 hours and a crew of 2 for 7 hours in the laboratory after sampling.

Table 2. Change in gene frequency when screening 4,000 males each generation.

Returning Adults	Gene Frequencies		Genotype Frequencies			No. of Males to be Screened	No. of Selected Eggs
Year	100	200	100/100	100/200	200/200		
1983	.892	.108	.796	.187	.017	4,000	4,569,600
1985	.842	.158	.718	.245	.037	4,000	6,316,800
1987	.778	.222	.620	.314	.066	4,000	8,512,000
1989	.700	.300	.510	.385	.108	--	--

schemes for increasing the AGP(200) frequency in Tutka Lagoon pinks. Table 1 shows the changes in gene frequency that would result if 25% or 50% of the eggs are fertilized with selected males. If the number of males screened is held constant, the gene frequencies would change as in Table 2--an intermediate between 25% and 50%. The third generation of selection in any of these methods can be used to achieve a desired variant frequency in the hatchery population. The intensity of selection would depend on the level of variant frequency. This frequency would be determined statistically after all major populations of pink salmon in the lower Cook Inlet, Kachemak Bay area were thoroughly screened. An estimate of the man-hours involved in the screening is given at the bottom of Table 1. The number of males that must be screened at either level of selection is not unreasonable. Based on this, a genetic marking plan for Tutka pink salmon is possible.

The value of a genetic tag in Tutka Lagoon pinks cannot be ascertained until all other area populations are analyzed. The larger AGP(200) frequency difference we can make, the better an estimate of contribution we will get. If the hatchery population is readily identifiable, then some of the biological interactions between hatchery and wild stocks can be examined.

#### REFERENCES

- Aspinwall, N. 1973. Inheritance of  $\alpha$ -glycerophosphate dehydrogenase in the pink salmon, Oncorhynchus gorbuscha (Walbaum). Genetics 73:639-641.
- Harris, H. and D. A. Hopkinson. 1977. Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Co., Amsterdam.
- May, B. 1975. Electrophoretic variation in the genus Oncorhynchus. The methodology, genetic basis, and practical applications to the fisheries research and management. M.S. Thesis, University of Washington, Seattle.

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