

Genetic Monitoring of Pacific Salmon Hatcheries

ROBIN S. WAPLES, GARY A. WINANS, FRED M. UTTER,
and CONRAD MAHNKEN

*Northwest Fisheries Center
National Marine Fisheries Service, NOAA
2725 Montlake Blvd. East
Seattle, WA 98112*

ABSTRACT

In the last few decades, and in response to substantial reductions in the abundance of wild populations of Pacific salmon, an enormous amount of resources in both Asia and North America has been devoted to artificial propagation programs. Several factors increase the possibility of rapid (often detrimental) genetic change in cultured populations, but genetic considerations are often overlooked in the effort to increase short-term productivity. Here, we discuss recent studies using electrophoretic data for chinook salmon, *Oncorhynchus tshawytscha*, that address three important concerns for hatchery populations: levels of genetic variability, stability of allele frequencies, and genetic interactions (due to straying or overplanting) between hatchery and wild populations. Results indicate that although there is no evidence for a general reduction in levels of genetic variability in hatchery stocks relative to wild populations from the same geographic area, allele frequencies over a period of one generation changed much more in samples from hatchery populations in Oregon than in nearby wild populations. The genetic changes in the hatchery stocks appear to be due to a combination of two factors: genetic drift due to reduced effective population size, and (in some cases) the infusion of genes from other populations through straying or transfer of broodstock between hatcheries.

Introduction

As a consequence of increased fishing pressure, loss of spawning habitat, and blockage of migratory routes, returns of wild anadromous salmonids in the Pacific Northwest have declined substantially in this century. In part to mitigate these losses, an extensive public hatchery system has been developed during the last several decades. Throughout most of this period, management practices at the hatcheries have been dictated primarily by production demands, and relatively little consideration has been given to the genetic quality of released fish and their effects on wild fish. The availability of large amounts of data produced by protein electrophoresis over the last decade has made possible a critical evaluation of the genetic status of Pacific coast hatchery populations of salmonids. Here, we summarize results from several recent studies which are pertinent to three important concerns: 1) levels of genetic variability found in hatchery and wild populations; 2) stability of allele frequencies in hatchery and wild populations; and 3) genetic interactions (due to straying or overplanting) between hatchery and wild populations.

Materials and Methods

The electrophoretic data discussed here were collected over the last decade at the National Marine Fisheries Service laboratory in Seattle. A considerable database exists for all the North American species of Pacific salmon, *Oncorhynchus*, but here we consider only data for chinook salmon, *O. tshawytscha*; for this species, data are available for populations from California to Alaska. Whole juvenile fish or tissue samples (muscle, liver, eye, heart) from adult fish were collected in the field and stored at -70°C until analyzed. Starch gel electrophoresis was performed as described by Aebersold et al. (1987). Each sample was surveyed for genetic variation at up to 100 presumptive gene loci, and genotypes inferred from the phenotypic banding patterns (see Utter et al. 1987 for discussion) were used to compute allele frequencies and a variety of standard indices of genetic variability and differentiation.

Levels of Genetic Variability

Recent policy statements (e.g., Northwest Power Planning

Council, 1987) regarding anadromous salmonids express two major concerns: that existing levels of genetic diversity be maintained, and that unique gene pools be preserved. Loss of genetic variability is a real concern for managed populations because constraints on money, space, and other resources often limit the size of the breeding population. In a closed population, approximately $1/2N_e$ of the existing genetic variation is lost each generation, with N_e being the effective number of breeders (Crow and Kimura 1970). The effective population size (N_e) is less than the actual number (N) if the sex ratio is uneven or if the variance in reproductive success among families is large—both factors that might be influenced by hatchery management procedures. Furthermore, if population size changes over time, long-term N_e is determined primarily by the effective number of breeders in the generation(s) with smaller size. Therefore, a population bottleneck (reduced effective breeding size in one or a few generations) can contribute appreciably to the long-term erosion of genetic variability.

To determine whether these effects are important in Pacific salmon, we examined two measures of genetic variability (average heterozygosity and effective number of alleles per locus) in a series of hatchery and wild populations of chinook salmon. The occurrence of consistently lower levels of genetic variability in hatchery stocks would suggest that artificial propagation has caused population bottlenecks. The heterozygosity data, however, provide no evidence of the erosion of genetic variability in cultured populations of chinook salmon in the Pacific Northwest. In each case where data are available for a comparison (Fig. 1), hatchery and wild populations from the same area have very similar levels of heterozygosity. This result differs from that reported in a number of studies of Atlantic salmon, *Salmo salar*, and rainbow, *Oncorhynchus mykiss*, cutthroat, *O. clarkii*, and brown trouts, *Salmo trutta* (review, Allendorf and Ryman 1987); some cultured populations of these species have been found to have greatly reduced levels of heterozygosity relative to the ancestral wild stocks.

Some interesting trends are apparent in the heterozygosity data for chinook salmon but these relate to geographic differences rather than to differences between hatchery and wild populations. In the Columbia River basin, coastal populations have higher heterozygosity than do lower river populations, which in turn retain more genetic variability than Snake River populations from farther upstream (Fig. 1). Populations from the Klamath and upper Fraser rivers also show reduced levels of genetic variability relative to those closer to the coast (Georgia Strait, Puget Sound). Presumably, these differences reflect the essentially independent evolutionary histories of the different areas and, perhaps, the smaller population size or increased frequency of population bottlenecks in the upper river populations (Winans 1989).

One drawback to the above analysis is that average heterozygosity is not very sensitive to the presence or absence of uncommon alleles. Although they contribute little to the measurement of heterozygosity, such alleles are potentially very important to a population because they allow a greater degree of plasticity in response to changes in the environment. The presence of numerous alleles (even those at low frequency) in a population ensures that each generation, many genotypic combinations are produced upon which natural selection might act. Because alleles at low frequency are easily lost if the effective breeding size is small, the average number of alleles per locus is a more sensitive indicator than average heterozygosity of undesirable changes in the genetic makeup of a population. According to Utter et al. (1989), the average number of alleles per locus for seven hatchery and six wild populations from Oregon were similar (1.74 and 1.68, respectively). This lends additional support to our conclusion that the wholesale reduction of genetic variability reported in some hatchery populations of *Salmo* (e.g., Stahl 1983) has apparently not occurred in chinook salmon hatcheries in the Pacific Northwest.

This result is encouraging, but by no means constitutes a clean bill of health for hatchery populations. If the genetic makeup of the source populations is to be perpetuated as accurately as possible, it is important not only to conserve overall levels of genetic variability, but also to avoid large changes in frequency of the alleles present. For example, consider a locus with two alleles (A and a), sampled in a population at two times, with the following frequencies observed—time 1: A = 0.8, a = 0.2; time 2: A = 0.2, a = 0.8. Hardy-Weinberg expected heterozygosity ($2Aa = 0.32$) remains unchanged, but allele frequencies have shifted drastically. Clearly, it is also important to monitor allele frequencies over time in artificially propagated populations.

Temporal Stability of Allele Frequencies

To evaluate the temporal stability of allele frequencies, we examined electrophoretic data for 21 coastal chinook salmon populations from Oregon and California that were sampled in each of two years (Waples and Teel 1990). For each population, allele frequencies in the two samples were compared at an average of 10 polymorphic loci. For each locus, a contingency chi-square test was used to test the hypothesis that the population frequencies were unchanged. Results of these tests are very revealing (Table 1). For the three California hatchery and the nine Oregon wild populations, the number of single locus tests showing a significant change in allele frequency ($1/16 = 6\%$ to $7/88 = 8\%$) was close to the value (5%) expected to arise from sampling error, while the figure for the nine

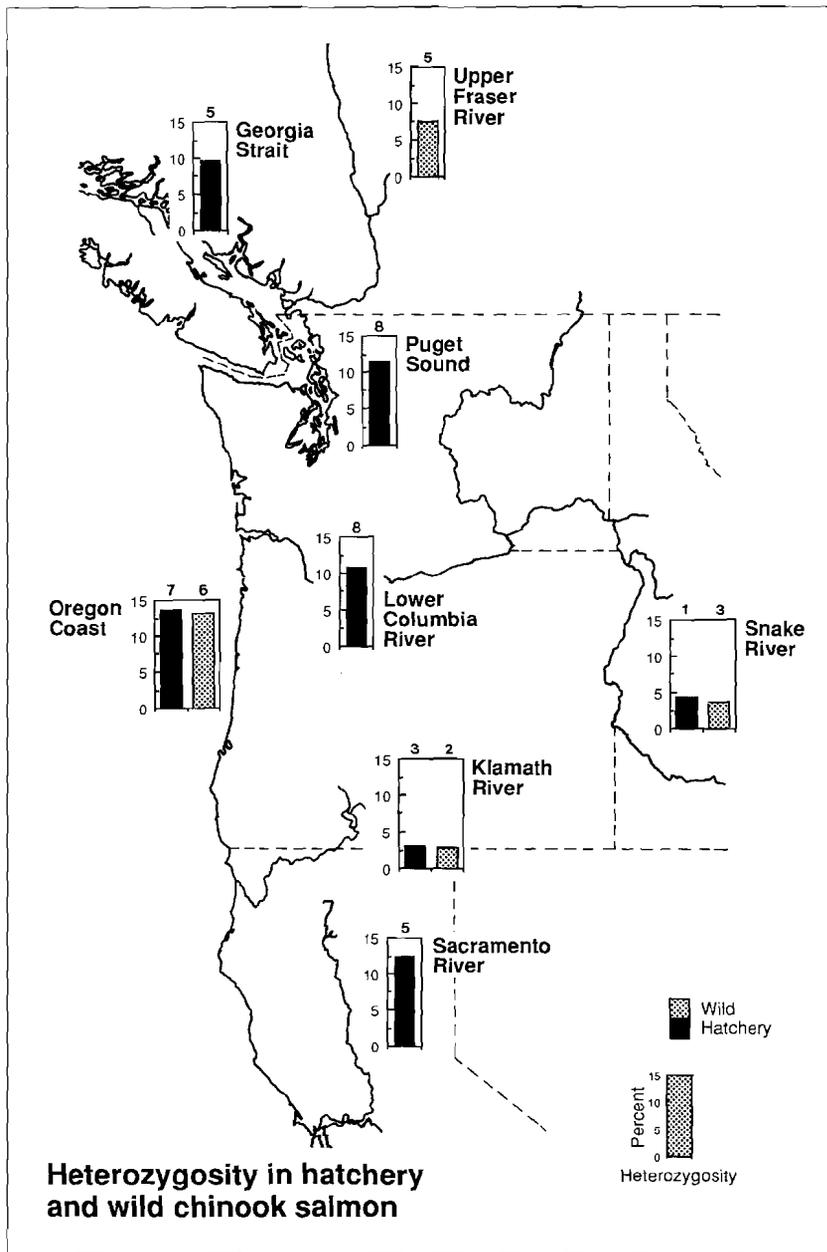


Figure 1

Comparison of average heterozygosity values for hatchery and wild stocks of chinook salmon by geographic area.

Oregon hatchery populations was much higher (29/81 = 36% of all tests showing significant allele frequency change). In addition, combined chi-square tests over all loci indicate very significant ($P < 0.01$) or highly significant ($P < 0.001$) changes in allele frequency between 1981 and 1985 samples in eight of the nine Oregon hatcheries (Table 1).

Possible causes of short-term allele frequency change include natural selection, genetic drift, and migration. In the present example, selection appears to be an unlikely cause, given Waples and Teel's (1990) demonstration that it is necessary to invoke unrealistically large selection coeffi-

cients to explain such large frequency shifts in a single generation. Waples and Teel also showed that the observed differences can be accounted for by genetic drift only if the effective number of breeders in the Oregon hatcheries averaged about 50 or less. Examination of brood stock data indicates that effective population size may indeed have been quite low in at least some of the hatcheries (Waples and Teel 1990).

Another possibility is that some of the genetic changes resulted from the infusion of new genes during the transfer of fish between hatcheries, or from natural straying into the hatcheries. Evidence to support this hypothesis comes

Table 1

Twenty-one chinook salmon populations sampled in each of two years. Number of significant ($\alpha = 0.05$) single locus chi square tests comparing allele frequencies in two years are shown, and significance levels are given for combined chi-square test over all loci and a test for gametic disequilibria (n.s. = not significant).

| Population ^c | Sample size | | Between-year comparisons ^e | | Gametic disequilibria ^d | |
|--|-------------|-------------------|---------------------------------------|----------|------------------------------------|-------------------|
| | 1981 | 1985 ^f | Single locus (no. sig./total) | All loci | 1981 | 1985 ^g |
| Oregon wild populations | | | | | | |
| Alesea | 94 | 50 | 0/11 | n.s. | n.s. | n.s. |
| Chetco | 100 | 93 | 1/7 | n.s. | n.s. | n.s. |
| Coquille | 115 | 50 | 1/12 | n.s. | n.s. | n.s. |
| Nehalem | 141 | 50 | 1/9 | n.s. | n.s. | n.s. |
| Nestuca | 60 | 50 | 1/9 | n.s. | n.s. | n.s. |
| Siletz | 92 | 50 | 0/11 | n.s. | n.s. | n.s. |
| Sixes | 100 | 50 | 1/8 | 0.01 | n.s. | n.s. |
| Siuslaw | 82 | 34 | 1/11 | n.s. | n.s. | n.s. |
| Tillamook | 88 | 50 | 1/10 | n.s. | n.s. | n.s. |
| No. sig./total | | | 7/87 | 1/9 | 0/9 | 0/9 |
| Oregon hatchery populations | | | | | | |
| Cedar Creek | 99 | 100 | 4/9 | 0.001 | 0.01 | 0.001 |
| Cole R. (S) | 113 | 50 | 1/9 | n.s. | n.s. | n.s. |
| Cole R. | 50 | 100 | 5/13 | 0.01 | n.s. | n.s. |
| Elk R. | 100 | 100 | 2/9 | 0.001 | n.s. | n.s. |
| Fall Creek | 100 | 100 | 2/7 | 0.01 | 0.001 | n.s. |
| Rock Creek (S) | 100 | 100 | 4/9 | 0.001 | 0.05 | 0.001 |
| Salmon | 99 | 100 | 5/8 | 0.001 | n.s. | 0.001 |
| Trask (S) | 100 | 100 | 3/10 | 0.001 | n.s. | 0.05 |
| Trask | 100 | 100 | 3/7 | 0.01 | 0.01 | 0.001 |
| No. sig./total | | | 29/81 | 8/9 | 4/9 | 5/9 |
| California hatchery populations | | | | | | |
| Iron Gate | 99 | 50 | 1/8 | n.s. | n.s. | n.s. |
| Trinity (S) | 50 | 100 | 0/5 | n.s. | — | 0.05 |
| Trinity | 100 | 50 | 0/3 | n.s. | n.s. | n.s. |
| No. sig./total | | | 1/16 | 0/3 | 0/2 | 1/3 |

^aSpring run denoted by (S); all others are fall run stocks.

^bSamples taken in 1983 for Oregon wild populations, 1984 for California populations.

^cData from Waples and Teel (in press).

^dData from Waples and Smouse (1990).

from gametic disequilibrium analysis, a powerful means of detecting samples which are actually a mixture of distinct gene pools. Gametic disequilibrium (the non-random association of alleles at different gene loci) occurs as the result of a mixture of gene pools that differ in allele frequency at two or more loci (Nei and Li 1973).

Genetic Interactions Between Hatchery and Wild Populations

Admixtures (mixtures of fish from more than a single gene pool) involving hatchery populations are a concern for two

reasons. First, the transfer of eggs, fry, and brood stock among hatcheries is a common occurrence that complicates the problem of identifying the genetic makeup of hatchery populations. Second, strays of hatchery or transplanted fish may have an adverse effect on wild populations adapted to local conditions. The genetic consequences of such admixtures are difficult to evaluate by traditional methods (physical tags, behavioral observations) because the presence of exotic fish in a population does not ensure that they will interbreed with the residents and produce viable offspring. If the potential source populations can be identified and adequate genetic markers are available, estimates of the mixture fractions are possible (Campton 1987). However, in many cases the populations possibly contributing

to a mixture are unknown or cannot be characterized genetically. For such a "blind" mixture, gametic disequilibrium analysis is a potentially powerful tool for evaluating the null hypothesis that the sample could have come from a single gene pool.

To evaluate the possibility of genetic admixture in the above example, we used a multilocus analysis of gametic disequilibrium (Waples and Smouse 1990) that considers data for all pairs of loci simultaneously. No unusual levels of multilocus gametic disequilibrium were found in the California hatchery or Oregon wild populations (1 of 23 samples with significant disequilibria at $\alpha = 0.05$ level; Waples and Smouse 1990), but the situation was quite different in the samples from the Oregon hatcheries (9 of 18 tests significant; Table 1). These findings are consistent with the hypothesis that, in addition to genetic drift, a mixture of gene pools contributed to the changes in allele frequency observed in some of the Oregon hatchery populations.

Gametic disequilibrium analysis has considerable potential for assessing the extent of genetic interactions among hatcheries and between hatchery and wild populations. It may help in the identification of wild gene pools that have been relatively unaffected by genes from hatchery populations and therefore merit conservation efforts. In other cases, where the objective is to supplement and enhance wild production, gametic disequilibrium analysis provides a means of monitoring the effectiveness of transplants from the hatcheries. The possibilities for both types of genetic interactions, as well as the necessity for monitoring them, are likely to increase in the near future. Large scale supplementation of wild populations and expansion of hatchery production are planned to achieve the goal of the current Columbia River Basin Fish and Wildlife Program (Northwest Power Planning Council 1987)—doubling the run size of anadromous salmonids in the Columbia River basin.

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