Alaska Department of Fish and Game
Genetic Policy
by
Genetic Policy Review Team

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INTRODUCTION

Alaska's valuable salmon industry relies on production from wild systems and, increasingly, on fish produced by aquaculture programs. The importance of maintaining healthy wild stocks and implementing successful enhancement activities underlies the need for an effective genetic policy. The genetic guidelines created to steer Alaska's aquaculture efforts were established in the mid-70's and have been reviewed to ensure that they reflect current knowledge, and goals. A revised genetic policy has been established that contains guidelines, supporting information and recommendations.

The genetic policy contains restrictions that will serve to protect the genetic integrity of important wild stocks. Certainly in Alaska where wild stocks are the mainstay of the commercial fishery economy, it is necessary to protect these stocks through careful consideration of the impacts of enhancement activities. Another important aspect of the genetic policy is the orientation towards increasing the productivity of enhancement programs in the state. Adherence to the guidelines will help maintain adequate genetic variability ensuring that the enhanced stock will be able to adapt to changing environmental conditions. The policy also includes considerations for selective breeding for desirable characteristics.

Due to the limited amount of information available on the genetic impacts of salmon enhancement on wild stocks, much of the basis for these guidelines is theoretical or based on work done with other species. Consequently, the most important considerations used in writing the guidelines are presented as a mechanism for illustrating the intent of the policy. An understanding of the rationale behind the policy is imperative to its effective application to individual cases under the very diverse conditions found in Alaska. The importance of the genetic guidelines will continue to increase as aquaculture activities expand their production. This policy represents a consensus of opinion and should continue to be periodically reviewed to ensure that the guidelines are consistent with current knowledge. By doing so, we will be able to meet the goal of greater fish production through enhancement while maintaining healthy wild stocks.

POLICY STATEMENT

I. Stock Transport

A. Interstate: Live salmonids, including gametes, will, not be imported from sources outside the state. Exceptions may be allowed for trans-boundary rivers.

B. Inter-regional: Stocks will not be transported between major geographic areas: Southeast, Kodiak Island, Prince William Sound, Cook Inlet, Bristol Bay, AYK and Interior.

C. Regional: Acceptability of transport within regions will be judged on the following criteria.

1. Phenotypic characteristics of the donor stock must be shown to be appropriate for the proposed fish culture regions and the goals set in the management plan.

2. No distance is set or specified for transport within a region. It is recognized that transplants occurring over greater distances may result in increased straying and reduce the likelihood of a successful transplant. Although the risk of failure affects the agency transporting the fish, transplants with high probability of failure will be denied. Proposals for long distance transport should be accompanied by adequate justification for non-local stock.

II. Protection of Wild Stocks

A. Gene flow from hatchery fish straying and intermingling with wild stocks may have significant detrimental effects on wild stocks. First priority will be given to protection of wild stocks from possible harmful interactions with introduced stocks. Stocks cannot be introduced to sites where the introduced stock may have significant interaction or impact on significant or unique wild stocks.

B. Significant or unique wild stocks must be identified on a regional and species basis so as to define sensitive and non-sensitive areas for movement of stocks.

C. Stock Rehabilitation and Enhancement

1. A watershed with a significant wild stock can only be stocked with progeny from the indigenous stocks.

2. Gametes may be removed, placed in a hatchery, and subsequently returned to the donor system at the appropriate life history state (eyed egg, fry or fingerling). However, no more than one generation of separation from the donor system to stocking of the progeny will be allowed.

D. Drainage's should be established as wild stock sanctuaries on a regional and species basis. These sanctuaries will be areas in which no enhancement activity is permitted except gamete removal for broodstock development. Use of such reservoirs for broodstock development should be considered on a case-by-case basis, and sliding egg take removal schedules applied to such systems should be conservative.

E. Fish releases at sites where no interaction with, or impact on significant or unique wild stocks will occur, and which are not for the purposes of developing, rehabilitation of, or enhancement of a stock (e.g., releases for terminal harvest or in landlocked lakes) will not produce a detrimental genetic effect. Such releases need not be restricted by genetic concerns.

III. Maintenance of Genetic Variance

A. Genetic diversity among hatcheries

1. A single donor stock cannot be used to establish or contribute to more than three hatchery stocks.

2. Off-site releases for terminal harvest rather than development or enhancement of a stock need not be restricted by III.A.1, if such release sites are selected so that they do not impact significant wild stocks, wild stock sanctuaries, or other hatchery stocks.

B. Genetic diversity within hatcheries and from donor stocks

1. A minimum effective population (Ne) of 400 should be used for broodstock development and maintained in hatchery stocks. However, small population sizes may be unavoidable with chinook and steelhead.

2. To ensure all segments of the run have the opportunity to spawn, sliding egg take scales for donor stock transplants will not allocate more than

90% of any segment of the run for broodstock.

GUIDELINES AND JUSTIFICATIONS

I. Stock Transport

A. Interstate: It is generally accepted that population of salmonids which have existed over many generations in a given watershed have evolved traits that make them adapted for survival in that environment. The greater the distance that a population is transferred from its native environment or the greater the difference in environmental conditions between the donor and stream, the less likely the genetic characteristics of the population will fit the new environment. If the fitness of the population is indeed reduced in the new environment, then the probability of the transport succeeding would be affected. In addition, interbreeding of a transferred stock with indigenous stocks could transfer gene traits that would reduce the fitness of the native populations. In many states, discrete stocks cannot be identified because excessive movement and interbreeding have already occurred. The State of Alaska, therefore, desires to protect and develop local stocks by restricting the movement of live fish or eggs into the state. There are, however, several trans-boundary rivers penetrating British Columbia, Canada, that flow into the state of Alaska. In some instances, donors from these stocks might fit a well-designed management plan.

B. Inter- Regional: The environment can vary greatly from one region to another in a state as large as Alaska. For similar reasons given in I.A. above, the transfer of fish from one region to another is restricted. Consideration may be given to regional border areas, especially when no suitable donor stock is available within a region.

C. Regional: Although it is recognized that indigenous stocks are best for donor stock development, there have been numerous successful transplants, especially if the environment at the new site is similar to that of the donor stock and distance between the sites is not great. There is insufficient scientific data to predict how far or how diverse the environment must be before a negative impact will occur. However, it is believed that within a region site matching opportunities may be available. As site matching characteristics decrease and transplant distance increases within the regional borders greater justification is required for the proposed transplant. The following should be considered when selecting a donor stock

1. Matching: Phenotypic characteristics of the donor stock should be matched to the environment at the site and to the management goals. Water chemistry and temperature profiles should be considered. Island stocks should be matched to other islands or to short rivers of comparable characteristics where possible. Time of spawning and fry emergence should be matched or compensated with the hatchery temperature required. Any

deviations should be addressed and justified in the permit application or the annual management plan.

2. Migration Routes: The probable migration routes and potential user groups should be identified. The applicant must determine a probable migration route based on the migration route of the proposed stock and characteristics (topography) of the transplant site. Coded wire tagging of hatchery releases can determine the accuracy of migration route predictions as well as assess possible impact on local stocks

II. Protection of Wild Stocks

A. <u>Prevention of detrimental effects of gene flow from hatchery fish straying and interbreeding with wild fish.</u>

Straying of hatchery fish released at the hatchery or off-station can potentially impact the fitness of wild fish populations through interbreeding of wild and hatchery fish. This assumes that hatchery and wild fish are adapted to different environments and either would presumably be less fit in the environment of the other and that hybrids would be less fit for either environment. Wild stocks have presumably been rigorously adapted to their native environment. Because of the large number of loci involved in the adaptation, many "successful" combinations of genetic information are possible along with the enormous number of "unsuccessful" combinations. Hybridization between discrete populations may produce a stock that has reduced fitness and therefore reduced production. Hatchery fish have been subjected to selection pressure for survival within artificial culture regimes, and may also have been originally derived from another stock adapted to totally different conditions than the impacted wild stock. Continued influx of hatchery fish together with the return of hybrids may alter the wild gene pool, reduce stock fitness, and thus threaten the survival of the wild population.

An alternative perspective is that hatchery strays will have little genetic impact on wild stocks. The influx of new genetic material through straying is a natural process in the development and expansion of salmon populations. If adaptation of the natural population is indeed very specific and selection is intense, then selection will favor and maintain the genetic complex of the wild populations. If adaptation is less specific and less intensive, then the genetic impacts from gene flow are insignificant. It is true that some straying occurs among adjacent wild populations and in most cases has occurred for a long enough time that such populations are quite similar genetically. However, situations in which transplanted stocks are involved are not analogous, as transplanted stocks would be less similar and gene flow would have a more profound effect. It is also true that the impact of introgression into the wild gene pool of genes from fish transplanted from a radically different environment may be limited by natural selection. Again the situations of concern do not necessarily lie near this extreme; hybrids and strays may be fit enough to dilute or replace the wild genome. Inherent homeostatic mechanisms for gene expression may compensate for some genetic influx.

The magnitude of straying relative to the size of the wild run is the most important criterion, as massive spawning by hatchery strays may jeopardize a wild population by displacement on spawning habitat and superimposition of redds, as well as, genetic influx. A conservative management approach dictates avoiding release sites where large numbers of hatchery strays can be expected to interact with significant or unique wild stocks. This approach can be achieved by spatial or temporal isolation of the hatchery and wild stock.

B. <u>Regional designation of significant and unique wild stocks.</u>

The magnitude of salmon populations varies between watersheds from intermittent runs maintained by straying to hundreds of thousands of fish. In evaluating the impacts of salmon enhancement projects, consideration must be given to the potential of detrimental effects from straying and intermingling with wild populations and possible resultant loss of wild production. Such consideration must take into account the benefits of the enhancement activity and the significance of the wild stocks impacted. Designation of criteria for runs of fish that are considered significant would greatly expedite the evaluation process. However, "significance" must be defined not only by the magnitude of the run, but also in the context of local importance and utilization. A small sockeye salmon stock near a village in southeast Alaska may be "significant", whereas the same size population may be too small to be considered a manageable entity in Bristol Bay. Because local utilization is an important concern, a regional planning group such as the Salmon Enhancement Regional Planning Teams, should consider what criteria will be used to determine significant stocks within a region and recommend such stock designations.

<u>Stock rehabilitation and enhancement.</u>

1. A watershed with significant wild stocks can only be stocked with progeny from the indigenous stocks. Rehabilitation of a watershed implies that there is insufficient production in habitat that formerly maintained a stock of some magnitude. Unless the indigenous stock has gone to extinction, use of an exogenous stock has potential for genetic damage noted in II. A. This damage will be exacerbated by the imprinting and homing of the transplanted stock to the impacted watershed, and potential displacement of wild juveniles by the exotics stocked in the rearing habitat. Enhancement of habitat not naturally accessible to salmon involves stocking eyed eggs, fry, or fingerlings, thus gaining production from this unutilized habitat. Where the inaccessible habitat is located above barriers on watersheds that maintain significant natural populations, stocking nonindigenous populations again has potential for genetic impacts noted in II.A.,

exacerbated by imprinting and homing of the transplanted stock to the watershed. For both rehabilitation and above barrier stockings, use of the indigenous stock alleviates these concerns.

2. When enhancing a stream using the indigenous stock, the fish used for stocking shall not be removed from the wild system to a hatchery for more than one generation.

   Hatchery incubation and rearing select for a limited set of biological and behavioral traits which are not necessarily the most suitable for survival in the wild environment. Because of this potential for such selection, the transfer of hatchery fish to rehabilitate or enhance stocks in depleted or underutilized watersheds runs the risk of altering the genetic character of the wild stock, even if the indigenous stock was the original donor stock for hatchery population. By restricting the separation between the transfer to the hatchery and the stocking to no more than one generation (e.g., eggs taken in a given year are cultured to fry or fingerling release at the hatchery; eggs or fish from the returns to the hatchery of this donor transplant are used for stocking), the risk of negative effects due to selection in the hatchery are minimized.

2. <u>Establishment of wild stock sanctuaries.</u>

   As noted in preceding sections, there is concern that hatchery culture of salmon through their freshwater (and in some cases, initial estuarine) life history phases may select for a limited set of biological traits that are not suitable for wild populations. Loss of genetic variability through intensive in-breeding for domestication and desired traits has often resulted in detrimental genetic effects in agronomy and agriculture, such as reduced resistance to disease or adverse environmental conditions. Original wild strains can provide the genetic variability needed to outbreed domestics and alleviate inbreeding depression. Because there is potential for detrimental impacts due to reduction of genetic variability, there is a need to preserve a variety of wild types for future broodstock development and outbreeding for enhancement programs. Designation of watersheds where hatcheries or hatchery plants are not allowed would allow wild stocks within these watersheds to be subjected to natural selection only, within the life history phases cultured at hatcheries. These watersheds would be "gene banks" of wild-type genetic variability.

III. Maintenance of Genetic Variance

1. <u>Genetic diversity among hatcheries</u>.

   There is general agreement that by introducing and maintaining a wide diversity of wild donor stock populations into the hatchery system that the prospects for long term success of the hatchery program in Alaska will be enhanced. Diversity tends to buffer biological systems against disaster, either natural or man-made. Developing and maintaining hatchery broodstock from a wide variety of donors will buffer the hatchery system against future catastrophes. Agricultural crop production in the U. S. provides a prime example of the dangers of genetic uniformity.

In an effort to increase yield, plant breeders have come to rely on a few highly productive strains. In 1970 approximately 15% of the corn production in the United States was lost to corn blight. The corn blight responsible, a mutant of the normal blight causing fungus, did not attack all strains. Only one strain of corn was vulnerable, but that strain of corn was grown by nearly every farmer in the country. Breeders were able to recover from the corn blight epidemic by replacing Texas cytoplasm with normal cytoplasm. Recovery was rapid because adequate genetic variability was available. There are other examples.

How does this relate to salmonid culture? Salmonid stocks apparently differ in levels of disease resistance, temperature tolerance, acid tolerance, and in their response to artificial selection. It seems imprudent to assume that conditions similar to those found in agriculture will not occur in aquaculture. In addition, the ability to genetically improve hatchery broodstock performance in the future will depend on the availability of genetic variability such as is found among wild salmonid stocks. A hatchery system with a variety of diverse broodstocks will be a valuable resource.

Genetic diversity does not guarantee protection from disaster, but uniformity seems to invite catastrophe. Local failures are inevitable within the hatchery system. It seems prudent to provide the system with a level of insurance by developing and preserving diversity among hatcheries.

Off-site releases for terminal harvest, whether for the commercial fishery or for a put and take sport fishery should have no adverse genetic effect if they are released at sites selected so that they do not impact significant wild stocks, wild stock sanctuaries or other hatchery stocks. The success of this type of release from a genetic standpoint depends on the ability to manage and harvest the return. If returns can not be harvested, increased straying may result which might lead to an impact on wild stocks at a greater than expected distance from the release site.

2. <u>Genetic diversity within hatcheries and from donor stocks</u>

There is a general consensus among geneticists that fitness (reproductive potential) is enhanced by heterozygosity (genetic variability). Any loss of genetic variation will be accompanied by a concomitant reduction in fitness. Genetic variation allows a population to adapt to a changing environment or to adapt to and colonize a new environment. Available genetic variation determines how rapidly a population will respond to either artificial or natural selection. On the other hand, selection, inbreeding and random genetic drift will reduce genetic variability in a population. Natural selection, that is selection for fitness, is a continuing process and should not be so intense that it has a significant effect in reduction of genetic variation, unless the population is in a new and quite different environment. Artificial selection on the other hand can be very intense, but can either be avoided or designed to assure that possible negative effects to fitness are offset by increased production efficiency due to the selection program, and by more efficient culture techniques. Inbreeding due to the deliberate mating of related individuals can be easily avoided in salmon hatcheries. Undoubtedly, in hatcheries and possibly in natural stocks the most important cause of loss of genetic variation is random genetic drift. In hatcheries reduction of genetic variation caused by inbreeding and genetic drift can easily be avoided by using adequate numbers of spawners.

Random genetic drift in general refers to fluctuations in gene frequency that occur as a result of chance. Such fluctuations occur, especially in small populations, as a result of random sampling among gametes. The amount of change but not the direction of change, can be predicted. The rate of this change is related inversely to effective population size (N_e). The smaller the effective population size the greater the fluctuation in gene frequencies. In small populations random genetic drift can result in inadvertent loss of genetic variability which may significantly reduce the fitness of the population.

Effective population size (N_e) is defined as the size of an idealized population that would lose genetic variability at the same rate as the sample population. An idealized population is one in which there is no mutation or selection, there are equal numbers of males and females, mating is random, etc. Obviously it is very unlikely that any natural population will meet all criteria for an idealized population.

Breeding structure of a population can profoundly affect the rate at which genetic variability is lost. However, we can determine the effective breeding size (Ne) for breeding structures and obtain the rate of inbreeding (ΔF) as

$$\Delta F = 1/2N_e$$ ∆©/\

so the consequences of breeding structure can be related to the loss of variation.

Many breeding structure variations can influence the effective population size. Four seem likely to operate in a salmon hatchery population: (1) numbers of males and females in the breeding population; (2) unequal numbers in successive generations; (3) nonrandom distribution of offspring among families; and (4) overlapping generations. These are discussed in greater detail in Appendix A.

Any of these variations in breeding structure may have a marked effect on N_e Although it may be impossible to control or even to measure variation in family size it is important to keep in mind the relationship to effective population size. Breeding plans that would aggravate or increase the variation of family size should be avoided. The effect of overlapping populations is to increase the effective population number, in that individuals mating in different years contribute to greater diversity. For example, it would take a larger number of pink salmon each year to maintain $N_e = 400$ than it would sockeye salmon.

The factor having the greatest potential effect in the hatchery and over which we have most control is sex ratio. As the formula indicates (Appendix A) the effective population size is affected most by the numbers of the least frequent sex. It is important to consider this in the breeding plan. In salmon, because a male can be used to fertilize the eggs of a large number of females, there is a temptation to do so. This temptation should be moderated by the necessity to maintain an effective population size which will assure that adequate genetic variation is maintained in the population. A minimum effective population (N_e) of 400 should be maintained. At this size the rate of in-breeding will be 0.125 percent per generation which should not have a significant effect on the long term fitness of the population.

In some cases, for example with chinook and steelhead, small population size may be unavoidable. In such cases a plan should be developed to offset the effects of small population size by infusion of genes from a source outside the hatchery population, such as the original donor source. Help in designing these breeding plans can be obtained from the Principal Geneticist, FRED Division, (absorbed into Commercial Fisheries Division in 1994) Alaska Department of Fish and Game.

While developing hatchery stocks from wild donor sources it is important that the genetic variability in the donor stock be protected. Cropping of the early or late run segments of a donor stock can change the timing of that run, which will reduce genetic variability of the population and may be detrimental to the stock's prospects for long term survival. To prevent such selection, sliding egg take scales for donor stock transplants should allocate no more than 90% of any segment of a run for broodstock.

RESEARCH

The necessity for much of this policy arises from our ignorance of the genetics of wild salmon populations and the effects of their domestication in hatcheries. The policy is based more on extrapolation from other disciplines such as agriculture than from first-hand knowledge of our resource. As a result, the policy is a somewhat conservative interpretation of these data in order to assure the long-term viability of salmon populations. The Committee has identified several areas in which specific knowledge would clarify this policy and contribute to the effectiveness of salmon enhancement. The Committee encourages cooperative research efforts among the university, state, federal and private sectors directed toward the general areas listed below.

1. Development of performance profiles of hatchery stocks and potential for genetic improvement. Information about stocks kept in culture will be useful in several ways. If taken in a standard manner, the data will be useful in determining the extent of variability in the species and will aid in the choice of stock to be used for outplanting or transplanting. The information will also be helpful in maximizing the production of a particular facility.

2. Potential for genetic improvement of cultured stocks. A sequel to the cataloging of the variability within and among stocks will be to experimentally assess the potential for genetic improvement by selective breeding. To do this, it is necessary to determine the heritabilities for traits of interest, that is the part of the phenotypic variability present in a population which results from genetic (heritable) causes as opposed to environmental causes. Traits such as size of adults, age of return and various timing parameters are particularly interesting to industry. Application of artificial selection is responsible for the enormous advances that have been made in agriculture; the potential also exists in aquaculture.

3. Assessment of the effect of introgression of genes from hatchery fish into wild populations. To examine this effect, one must first have an estimate of the rate of straying and the factors that influence straying. Such factors might include transplant distance, run strength, source of the hatchery stock and year-to-year environmental differences. By using a genetically marked stock, one can monitor the flow of "hatchery genes" into other populations. Because the effect of such introgression may develop over time, it is necessary that such an experiment be conducted over several generations. For this kind of study, it may be necessary to develop a means for marking fish cultured at production levels.

   The second part of this problem is to establish the impact of introgression. A range of potential interactions is possible ranging from introgression between two unrelated stocks to the introgression of fish subject to the selective pressures of a hatchery back into the wild stock from which they were derived. Research to examine these effects could best be done in an experimental hatchery where hybrid stocks could be produced and all releases marked. Port sampling and stream walking would be necessary to evaluate survival, straying and other phenotypic effects.

4. The effects of inbreeding and maintenance of inbred lines. Accompanying the artificial propagation of a species is the potential for inbreeding, loss of genetic variability and increased homozygosity. Information pertinent to the extent of inbreeding depression that results from various levels of inbreeding is necessary in determining adequate effective population sizes. This is especially important for species for which a large effective population size is difficult to maintain. In

addition, this information would permit a judgement on the efficacy of enhancing very small remnant populations. This work could be done both by performing crosses designed to accomplish some level of inbreeding, and by the maintenance of small randomly breeding populations. In both cases, it is important to keep careful controls.

Appendix A

The relationship of breeding structure, effective population size, and rate of inbreeding.

Breeding structure can profoundly affect effective breeding size (N_e) of a population. We can, at least in theory, determine the effective breeding size for many breeding structures and obtain the rate of inbreeding (AF) as

$$AF = 1/2Ne$$

directly relating variation in breeding structure to loss of genetic variation. (See D.S. Falconer. 1981. Introduction to Quantitative Genetics. Longman Inc., New York.)
The following demonstrates the consequence of some breeding structures to effective population size.

Number of males and females: Unequal numbers of males and females in the breeding population reduce effective population size. Sex ratio is related to effective population number (Ne) as

$$Ne = 4NmNf/(Nm = Nf)$$

where Nm and Nf refer to the total number of males and females respectively. The effective population size is strongly influenced by the number of the least frequent sex.

Unequal numbers in successive generations: If the numbers of breeding individuals is not constant in successive generations the mean effective number is the harmonic mean of the number in each generation. Over generations the effective number is approximately,

$$1/Ne = 1/t(1/N1 + 1/N2 + 1/N3 + ...1/Nt).$$

The generation that has the smallest number will have the largest effect.

Nonrandom distribution of offspring among families: When there is large variation in family size the next generation is made up of the progeny of a smaller than expected number of parents. This can be related to loss of genetic variation through effective population number as

$$Ne = 4N/(Vk + 2)$$

where Vk refers to the variance in family size. When variation of family size Vk is equal to 2, then N = N. When the number of males and females are unequal, the variance of family size may be unequal in the two sexes and

$$Ne = 8N/(Vkm + Vkf + 4)$$

where Vkm and Vkf are the variance of family size for males and females respectively.

Overlapping generations: In species other than pink generations are not discrete, they are overlapping. When generations overlap the effective population size is

$$Ne = 4NcL/(Vkm + 2)$$

When where L is the generation time and Nc is the number of individuals born in a year, that is the cohort size. The cohort size Nc is related to the total number (Nt) by Nc = Nt/E and E is the mean age at death. As before Vkm is the variation of family size. The effect of unequal sex ratio and unequal numbers in successive generations on population size can be easily estimated. On the other hand it will be difficult or perhaps impossible to estimate the variance of family size. Nevertheless, we should keep in mind the relationships of family size and overlapping generations. Overlapping generations will in general increase the effective population number in that individuals mating in different years contribute to greater diversity. Variance of family size can radically reduce effective population size. Procedures that contribute to variance of family size or separation of year classes should be avoided.