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GENETIC INTERACTION OF AUKE CREEK HATCHERY PINK SALMON WITH NATURAL SPAWNING STOCKS IN AUKE CREEK

by

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The Auke Creek Hatchery and weir run by the National Marine Fisheries Service Auke Bay Laboratory was essential to the execution of this project. Dr. W.A. Smoker and Dr. G. Snyder, the Laboratory directors during the project generously made the facility available for this project and their personnel were accessible for advice especially Mr. S.G. Taylor, a collaborator on many aspects of this project. Supported by this project were graduate students A.J. McGregor and S. Lane. Also participating in various aspects of the project were M. Thomason, S. Shirley, and C. Smoot. Many other faculty members, graduate students, undergraduates, and friends were conscripted during times of crisis, not the least of whom were Dr. W. W. Smoker and my wife J.A. Gharrett. I appreciate the reviews of this report by T.E. Dowling, J.T. Gharrett, and J.A. Gharrett.

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INTRODUCTION

In Alaska, extensive salmonid hatchery programs have been initiated both by the Alaska Department of Fish and Game and by private-nonprofit organizations. It is intended that these hatcheries produce fish to augment, rather than replace natural production. The "wild" stocks are important not only for their production potential, but also as a source of genetic variation. To assure the continued existence of "wild" stocks it is necessary to continually assess the impact cultured fish have on "wild" fish.

Cultured fish may have various impacts on "wild" populations including pathological, ecological, and genetic. One particular impact can result from fishing on mixtures of hatchery and "wild" fish. Because of the enormous differences between the escapement goals of a hatchery and those of a natural system, managers must be very careful not to over-harvest the "wild" stocks. In order to avoid this kind of problem, an effective and economical means for uniquely marking the fish produced at these facilities is necessary. In addition to being useful for management, marking would be applied in evaluating the performance of each hatchery.

A more direct impact would result if hatchery fish were to stray into a "wild" system and interbreed with "wild" fish. Such a scenario is not at all unlikely in the vicinity of a hatchery where a large infusion of hatchery fish and subsequent over crowding of the hatchery stream may lead to increased straying of hatchery fish. No adverse affect would be expected from the introgression of hatchery genes into the "wild" population if the

two were genetically identical; however, if the two were genetically distinct, one would expect that the "wild" gene pool would eventually be replaced by the hatchery gene pool. The ultimate result of the latter situation would be the loss of genetic variability attributable to that "wild" population and possibly a decrease in production by a genetically less suited population. The rate of displacement by the hatchery gene pool and the magnitude of the loss of production are impossible to assess from the present knowledge of salmonid biology. In particular, the integrity of spawning populations, spatial and temporal, and the normal extent of straying among populations as well as the influence of hatcheries on straying, must be quantified before this kind of interaction can be assessed.

Planning for the extant hatcheries, as well as those not yet constructed, has attempted to minimize interactions between hatchery and "wild" fish; but the biological planning is influenced by economic factors. The conflict of biological and economic planning has often been detrimental to resources because the existing information on the biology of the resource is inadequate for economic evaluation or forecasting.

The project reported here was initiated to investigate the methodology and biological effect of a novel form of marking, genetic marking, and to use the markers to examine some aspects of the biology of a marked stock that would be useful in assessing interactions between hatchery stocks and "wild" stocks. Genetic markers were placed in both odd- (1979 and 1981) and even- (1980) year runs of pink salmon (Oncorhynchus gorbuscha) in the NMFS Auke Creek Hatchery in S.E. Alaska. The persistence of

the mark was monitored through 1984. Those results were used both to evaluate the genetic marker and to examine the precision of homing, both temporal and spatial, of the hatchery stock.

This report is not meant to be an exhaustive coverage of the project; rather, a vehicle for reporting the significant results and tying together the two M.S. theses (McGregor 1982, Lane 1984) and single publication (Gharrett et al. 1983) that have to date resulted from this work.

BACKGROUND

Genetic marking

Effective management of a fishery for an anadromous species requires knowledge of the origin(s) of the exploited stock(s). This knowledge can be obtained only if the fish possess some kind of marker that indicates their origin. Other information useful to managers that also requires marking fish includes life history and survival of fish stocks (Davidson 1934, Pritchard 1939), migration routes (Nakatani et al. 1975, Hoffman 1982), and run timing (Davis et al. 1979, Anon. 1980).

Methods which have been used to mark fish or to identify stocks include fin excision, coded micro-wire tags, and scale pattern analysis. All have both positive and negative aspects (Gharrett et al. 1983). Genetic marking, which has been advocated as an alternative to these methods for some applications, has the unique property that it is heritable (Utter et al. 1966, 1974,

and 1976; Utter and Hodgins 1972; Jamieson 1974; May 1975; Hedgecock et al. 1976; Moav et al. 1976 and 1978; Allendorf and Utter 1979; Krueger et al. 1981); that is, once it has been placed in a stock, it is passed on from generation to generation. There are several advantages to a heritable mark: the cost of marking need only be incurred once, the mark of a particular stock does not change from year to year, and the marked fish are not mutilated. In fact, the type of genetic trait used for genetic marking is not visible to the consumer.

The genetic "markers" that may be used most conveniently are a class of biochemical genetic traits (enzymes) detectable by starch-gel electrophoresis (eg. Utter et al. 1974). These traits are inherited in simple Mendelian fashion and exhibit considerable natural variability of expression (allozymes) within most species and even within most populations of those species. The variation in the genetic compositions of different groups (populations) of fish may be used to distinguish among those groups or to identify a particular group. In some situations, differences in genetic composition occur naturally among groups especially when those groups are reproductively isolated (eg. Utter et al. 1976). Where hatcheries exist, the genetic composition of the hatchery stock may be altered artificially thereby "genetically marking" it. Specifically, the frequency of a relatively rare allele at a biochemical genetic locus is increased in the population by selectively breeding individuals possessing that allele.

Biochemical genetic markers have been been used for a variety of purposes on various species of fish and other

organisms (Gharrett et al. 1983), but there has been no evaluation of the potential detrimental effects of altering the genetic composition of a population. The genetic composition of a population derives from genetic variation generated by mutation and molded by natural selection and random alterations (random drift). There is presently a controversy as to which of these processes is more instrumental in determining the genetic composition particularly with respect to traits such as biochemical genetic traits. Since the process of genetic marking alters the genetic composition of the "marked" population, it is important that the trait used for marking not be strongly influenced by selection.

Most published studies involving genetic marking either assume (often tacitly) that no selective disadvantage exists (i.e. the allele used for marking is "neutral") or (rarely) attempt to design the experiment to nullify effects of selection. To our knowledge, only one other study has attempted to to examine the assumption of neutrality (Chilcote et al. 1980 and 1981) and this study had difficulties in data procurement and interpretation. For short term experiments, the assumption of neutrality is probably reasonable because the marker allele would not exist naturally in the population unless a strong balancing mechanism similar to that observed for sickle-cell anemia in malaria infested regions was at work. For experiments spanning several generations (such as genetically marking a population of fish), the effects of a moderately deleterious allele would tend to reverse the change in genetic composition accomplished by the marking, that is, the frequency of marker

would gradually revert to its original level. Moreover, the decreased fitness of the stock would result in decreased production.

One of the reasons efforts have not been made to test the assumption of neutrality is that small differences in selection are quite difficult to quantify. The sample sizes necessary for such measurements increase approximately exponentially as the selection differentials decrease. Experiments performed for this project were designed to elucidate substantial (>10%) changes in fitness but also to set up a system in which selection (changes in genetic composition) could be monitored over future generations. Analyzing the changes over time should provide resolution of even smaller differences.

Genetic interaction between "wild" and hatchery fish

The introgression of genes from hatchery fish into a "wild" population whose genetic composition has been adapted to its environment will at best produce no effect on the "wild" population, and at worst replace the "wild" population with a stock maladapted to the natural environment with a substantial interim loss in production in the "wild" system.

One would expect no genetic impact if the two were very similar genetically, for example, if the hatchery stock were derived from the "wild" stock and hatchery practices had not led to alterations in the genetic composition (eg. inbreeding) of the hatchery stock.

In contrast, if the hatchery stock were derived from a source genetically distinct from the "wild" stock, the result would most likely not be the heterosis (hybrid vigor) obtained by crossing two highly inbred (homozygous) lines; rather, hybrid dysgenesis, a decrease in the fitness and resultant production by the hybrid types. Hybrid dysgenesis results from disruption of the unique combinations of coadapted genes that evolved independendently in each of the populations in response to selective environmental pressures, but which, as a result of segregation and assortment during gametogenesis, recombine in hybrids and their progeny and produce genotypes less fit than those of either original stock.

Genetic exchange between populations can be measured unequivocally only if quantifiable genetic differences exist between the populations. If such differences exist, introgression of genes from one population into the other can be monitored over time. Other methods such as the observation of fin-marked fish are only inferential because mere presence of marked adults on spawning grounds does not prove that gametes are contributed.

A genetic marker placed in a hatchery stock provides the quantifiable difference required for such a study. Therefore, in addition to placing a genetic marker in a hatchery population and monitoring the marker, we also monitored the frequency of the marker allele in other local populations of pink salmon spatially and/or temporally distinct from the hatchery stock. Straying of hatchery fish into these populations would cause subsequent increases in the frequency of the marker allele in affected populations. Of course, if the marker were substantially affected

by selection, it would be difficult to monitor straying.

Because there is natural production of pink salmon in Auke Creek and the small hatchery stock is derived from native pink salmon with no intention of maintaining a purely "hatchery" stock, the homing behavior of the genetically marked Auke Creek hatchery stock may more represent that of a "wild" stock than of a large hatchery stock, especially one derived from transplants. Regardless, information obtained from monitoring the frequency of the genetic marker in the local populations provides an indication of the discreteness of pink salmon populations and for this situation a minimum estimate of the extent of genetic interaction between hatchery and local "wild" stocks. Better estimates could be obtained only from "production" hatcheries.

APPROACH

The goals of this project were to place a genetic marker in a hatchery population, evaluate the marker and the marking process, and use the mark to obtain information on the integrity of salmon populations. A flow diagram is presented in Figure 1.We chose to work on pink salmon 1) because they have the shortest generation time (2 years) which would allow us to examine two generations of marking, 2) the artificial culture of this species is well studied, 3) the existence of two distinct, non-interbreeding runs (even-year and odd-year runs) in Auke Creek would permit two parallel experiments, 4) the substantial genetic variability that existed within the species provided a number of

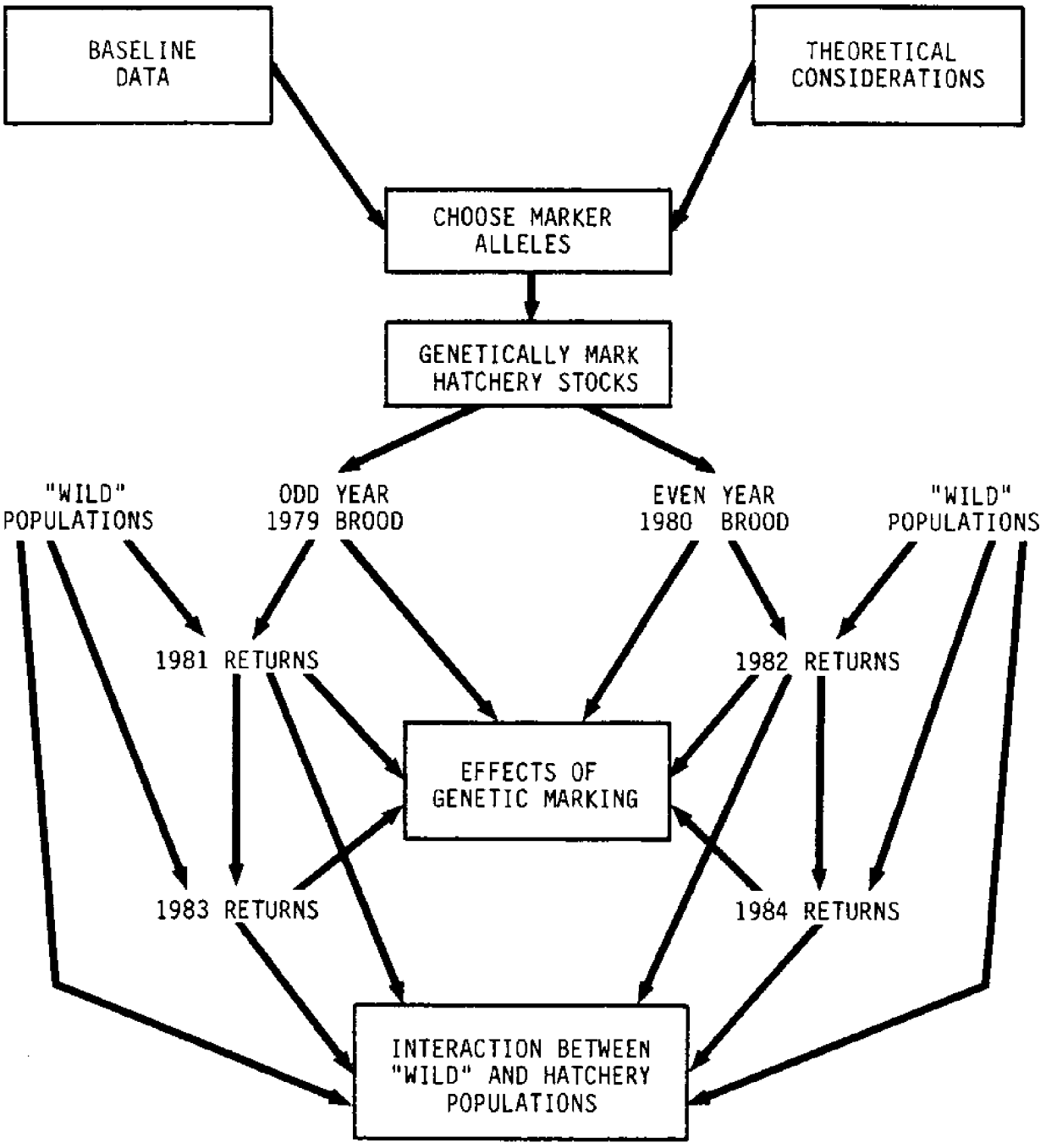

Figure 1. Flow chart for approach to genetic marking and examination of genetic interaction between hatchery and "wild" fish.

potential genetic markers, 5) the National Marine Fisheries Service Auke Bay Laboratory, which runs an experimental pink salmon hatchery, agreed to cooperate in this study, and 6) the hatchery was conveniently located on a well studied system.

Prior to genetically marking the hatchery population it was necessary to obtain some baseline information on the pink salmon populations in the area of the hatchery that might interact genetically with the hatchery stock. This information was needed to select an appropriate marker allele. Such an allele should be present at relatively low levels in the population from which hatchery brood stock was taken, have remained stable at that frequency for several previous generations, and not be present at elevated levels in any other local populations. Using these background data and taking into account the resources available for the marking effort, it was possible to choose the marker that would optimize our efforts.

The marking was accomplished by screening potential breeders for the presence of the marker allele and breeding only those possessing the marker. This process increases the frequency of the marker allele in the marked population.

Evaluation of the effect of the marker was accomplished by monitoring the frequency of the marker over two full generations: in a sample of outmigrating fry from the original marking, in the adults returning the following generation, in a sample of their fry, and in the adults returning the second generation. Decreases in the frequency would be indicative of detrimental effects by the genetic marking process or, less likely, of substantial straying of "wild" fish into the hatchery population. In the

first generation, a large portion of the genetically marked fry were also fin-clipped prior to their release to ensure their unequivocal identification upon return. Because Auke Creek also supports naturally spawning pink salmon only returning fin-clipped adults were used as brood stock the second generation in a second effort to elevate the frequency of the marker allele.

The temporal and spatial definition of the genetically marked hatchery population was followed by monitoring the frequencies of the marker allele in spawned-out fish sampled throughout the spawning period from three distinct regions of the Auke Creek system, as well as from another local stream, Waydelich Creek. Outmigrating fry were also analyzed. Increases in the frequency of the marker allele above the baseline levels of unmarked populations would be indicative of straying of hatchery fish into those areas, that is, genetic interaction with the "wild" fish.

SUMMARY OF RESULTS

Auke Creek Hatchery and Auke Lake stream system

The Auke Creek Hatchery (Figures 2 and 3), run by the National Marine Fisheries Service Auke Bay Laboratory in cooperation with the Alaska Territorial Sportsmen, was used for this project. Auke Creek is located approximately 18 kilometers northwest of Juneau, Alaska and flows approximately 350 meters from Auke Lake into Auke Bay. The system is small and readily accessible to our laboratory at the University of Alaska, Juneau.

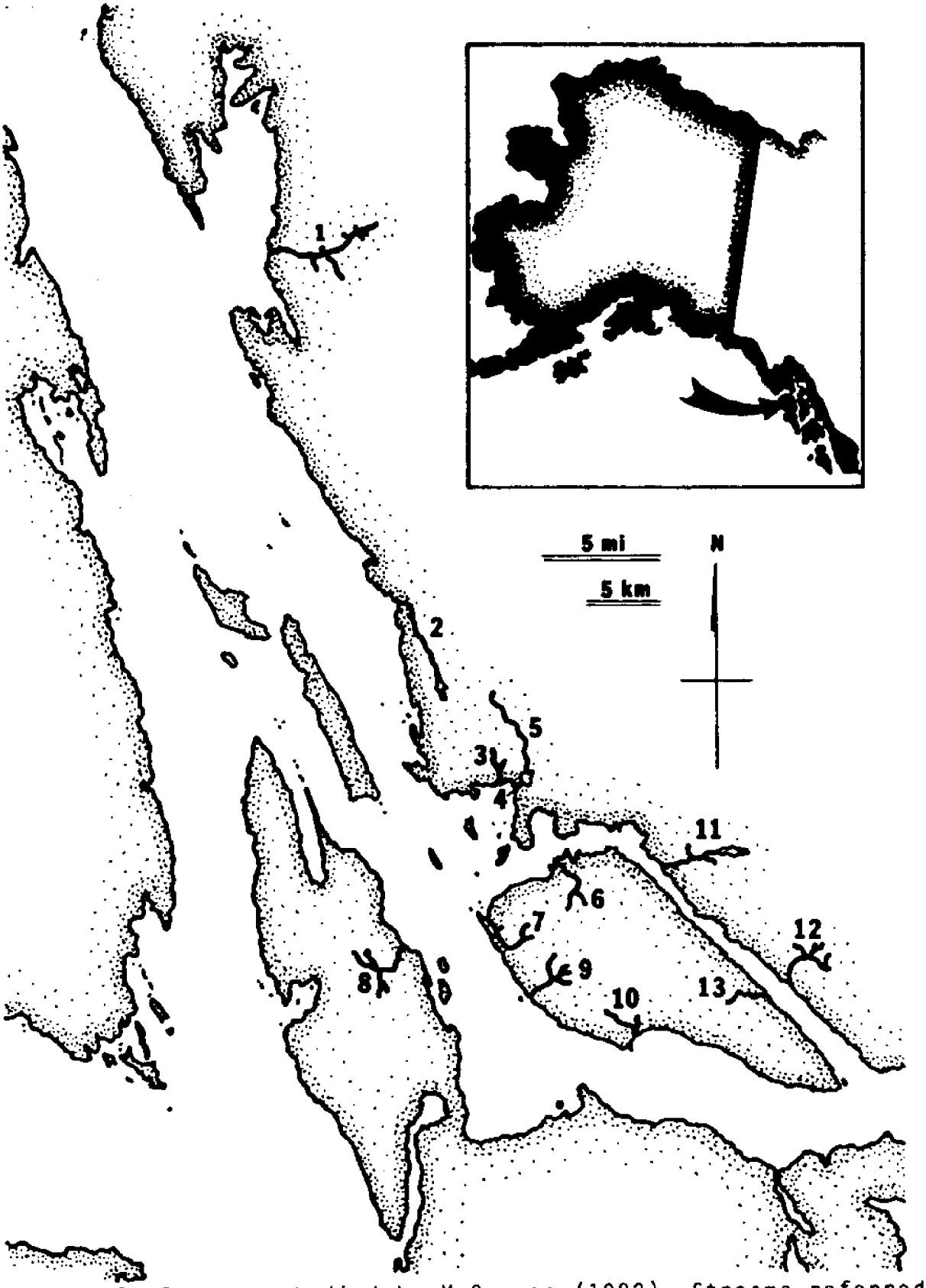

Figure 2. Streams studied by McGregor (1982). Streams referred to in this report are Auke Creek (4), Lake Creek (5), Waydelich Creek (3), and Fish Creek (6).

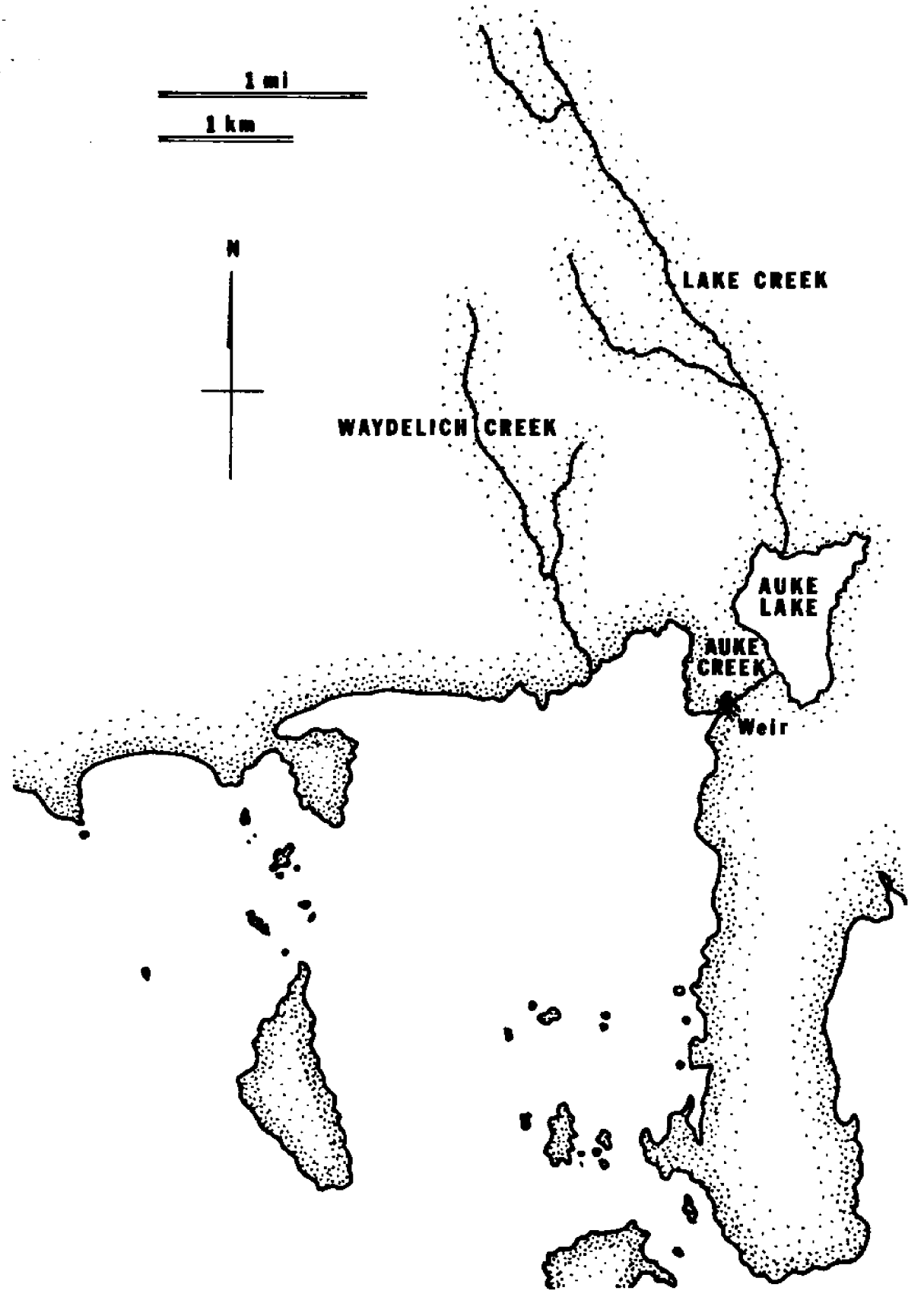

Figure 3. The immediate vicinity of the genetically marked Auke Creek Hatchery population.

The Auke Creek Hatchery is ideally suited for experimental genetic marking not only because of the existence of the incubation facility and elaborate fish counting weir, but also because 1) the salmonid populations of the Auke Lake system have been well characterized as a result of research done by the National Marine Fisheries Service and the State of Alaska Department of Fish and Game, and 2) the hatchery pink salmon stock is genetically the same as the "wild" pink salmon in Auke Creek since the hatchery brood stock has been taken at random from returning adults with no attempt to distinguish between hatchery and naturally produced fish.

Both even- and odd-year pink salmon populations occur in the Auke Creek system. In each year, there are several distinct breeding groups distinguished both by timing of spawning run and by the location of spawning within the system (Taylor 1980). An early run begins in late July and lasts through late August; a late run begins in late August and lasts through late September. Often there is little or no overlap between these two runs. Each of these runs segregates into two groups, one of which spawns intertidally, below the weir, the other above tidal influence, above the weir. The effect of the weir on the integrity of these breeding groups is unknown.

Baseline data

The first step in genetically marking a population is to genetically characterize the population to be marked as well as all other populations with which the marked population is likely

to interact or from which it is to be discriminated. With these data it is possible to choose a marker allele that is unique, at least within the geographical range scrutinized. In addition these data permit the examination of the stability of allelic frequencies within populations and provide the background information necessary for studying the interaction of the hatchery population with other populations.

The thesis work of A.J. McGregor (1982), a part of this project, provided the baseline data necessary for marking the Auke Creek Hatchery population. Using starch-gel electrophoresis of 25 biochemical genetic loci, McGregor characterized the genetic compositions of pink salmon populations in the vicinity of Auke Creek for both even- and odd-year pink salmon (Figure 2). In order to determine the extent of genetic variability that could be expected for pink salmon, he compared these data to other data he obtained from populations further removed, including ones in southern southeastern Alaska and as far away as western Alaska.

The genetic compositions of the populations near Auke Creek, while not completely homogeneous, were quite similar. For genetic marking purposes this meant that inter-populational differences were of no concern in the selection of a marker allele. At the population genetics level, this tendency toward homogeneity among populations suggests that these populations are descended from a common ancestral population and that any divergence resulting from processes such as natural selection or random genetic drift is mostly reversed by a low level of straying (gene flow) among these populations, at least for the traits examined.

The stability of allelic frequences over time was established by comparing our results from studies made in 1978, 1979, and 1981 on pink salmon populations in Fish Creek, a stream in the study area, to data previously reported by Aspinwall (1973 and 1974a) for samples also taken from Fish Creek in 1969-1971. The frequencies of the three less common alleles at the loci studied did not change substantially during the generations represented in the two studies, indicating that the frequencies observed in a population were, essentially, in Hardy-Weinberg equilibrium. Practically, this meant that one might expect a marker to persist in a population and that one need not expect random fluctuations of the marker allele in other populations that would decrease the discriminatory value of the marker allele.

Other results showed that substantial genetic divergence existed between the more distal populations indicating little or no gene flow between these populations. Most interesting of these comparisons was the observation that more divergence had occurred between even- and odd-year runs within the same stream system than between the most geographically distant populations in the same year. For example, there are more genetic differences between 1979 and 1980 pink salmon runs within Auke Creek than there is between 1980 adult returns to Norton Sound and to Southeast Alaska, which are separated geographically by several thousand kilometers of water. These differences in genetic composition indicate that virtually no gene flow occurs between even- and odd-year pink salmon and suggest that the differences that exist within a stream are the result of random genetic drift

(Aspinwall 1973, 1974ab).

In addition to obtaining a baseline for the genetic marking process, McGregor (1982) also performed numerous crosses to confirm the Mendelian nature of the biochemical genetic traits used to characterize the populations.

Theoretical considerations in choosing a genetic marker

The perfect genetic marker would have a frequency of one in the marked population and be absent in all other populations. Unfortunately, marking a population with a mark of this quality is not practical. Aside from the question of the source of such a marker allele, a number of other factors influence the marking process. These factors may be divided into genetic, biological, and economic categories.

Genetic factors that must be considered include the genetic composition of the population to be marked relative to those of other local populations (information available from the baseline) and the nature of inheritance. Being diploid, every salmonid carries two copies of each gene, one copy received from each parent. These copies need not be absolutely identical, hence the existence in a population of different alleles for a particular trait. If two alleles (A and A') exist for a particular gene (at a locus) in a population, different individuals within the population may carry either one or both of the possible alleles. Three different types (genotypes) of individual would be possible AA, AA', and A'A'. If A' were the less common allele chosen for genetic marking, it is clear that the marking process would be

more efficient if one used only A'A' individuals. However, probabilistically the A'A' individuals would be the least frequent type in the population; in fact, for low frequencies of A', the A'A' types may be virtually nonexistant in the population. In this case, AA' individuals would be the only source of A' alleles. The initial frequency of the marker allele in the population being marked and the diploid nature of inheritance must be considered in the marking process.

Timing, duration, and size of the run must all be considered in genetic marking. These biological factors determine the amount of "raw material" from which the marked population can be taken and the time it is available. Because the genetic marking process involves breeding only a fraction of the potential spawners (those carrying an appropriate complement of marker allele), a compromise must be made between the size of the marked stock and the extent to which the frequency of the marker allele is increased. The genetic "health" of the marked population also requires minimal loss of genetic variation at loci other than the marker locus. This can be assured only if reasonable numbers of both males and females are used in the crosses performed to mark the population. Genetic guidelines of the Alaska Department of Fish and Game recommend an effective population size (N_e) (Falconer 1981) of 400 individuals. One way to achieve this number is to breed 200 females and 200 males. Maintaining a reasonable effective population size also affects the marking strategy.

Overlying these concerns are economic considerations, notably the resources available to perform the marking. Some

combination of resources, run size, and available marker alleles will restrict the marking effort. Given restrictions, it is important to maximize the effectiveness of the mark. We (Gharrett et al. 1983) have derived equations that may be used to choose (given restrictions of manpower, budget, egg-take goals, and run size) marker alleles that would maximize the ability to discriminate the marked population from unmarked populations.

Implementing the genetic mark

The thesis work of S. Lane (1984), supported by this project, described genetic marking experiments and evaluated the marking procedure and the effect of the marker on the hatchery population, then, examined the temporal and geographical bounds of the hatchery population in an attempt to determine the extent of genetic interaction between hatchery and "wild" fish.

Two different strategies were chosen for genetically marking the odd- and even-year runs of pink salmon in Auke Creek. The particular breeding population of Auke Creek that served as brood stock for the hatchery for both runs were the late, upstream spawners. The strategy for the odd-year run was chosen to maximize the effectiveness of the marker given the run size and available effort. The particular allele chosen for marking was a slowly migrating electrophoretic variant (allele) at a malate dehydrogenase locus (MDH-3,4). In pink salmon, the MDH loci are duplicated, which means that multiple identical (or very similar) genes exist in pink salmon that specify the same enzymatic end product. We felt that the existence of these multiple genes would

buffer any potentially deleterious effects engendered by the alteration of the genetic composition of MDH loci.

The strategy adopted for the even-year run was to nearly fix a less common allele of the alpha-glycerophosphate locus (AGP). While this strategy did not maximize the effectiveness of the marker for discriminating the marked population from the other populations, it did maximize our chances for observing decreases in fitness related to the genetic marker, one of the objectives of this project.

The odd-year marking was accomplished by screening 3,906 adult pink salmon returning through the Auke Creek weir in mid September, 1979 for fish either heterozygous or homozygous for the slow MDH-3,4 allele. Fish were screened by taking a small tissue sample for analysis, marking sampled fish with uniquely numbered tags cross-referenced with the tissue samples, and holding sampled fish until the tissues had been analyzed. The 407 fish that were determined to possess the marker allele were used for hatchery stock. During the marking process, it became apparent that we could also easily remove a fast migrating allele from the population, thereby doubly marking the hatchery stock. The marking process resulted in increasing the allelic frequency of the slow MDH allele from 0.054 to 0.508, nearly ten-fold, and decreasing the frequency of the fast allele from 0.046 to 0.00, extinction. Fish not suitable as brood stock for genetic marking were released to the stream.

One of the goals of this project was to compare the allelic frequencies of the parents of the genetically marked population to those of their progeny returning the next generation;

therefore, prior to release, 60,000 of the 178,219 genetically marked fry were also fin clipped so they could be unequivocally recognized at return.

In 1980 the marking of the even-year run was accomplished by screening 7,710 late run pink salmon for a fast migrating AGP allele and spawning 396 fish, 303 possessing two doses of the allele and 88 possessing a single dose of the allele. Inadvertently, 5 fish not possessing this allele were included as spawners. The marking effort succeeded in increasing the frequency of the marker allele from 0.199 to 0.891. For this experiment, 85,747 of the 175,827 genetically marked fry were also fin marked.

The genetic marking process requires a crew of four people to sample the fish and one person to perform the electrophoretic analysis. With this manpower and sufficient fish, between 500 and 700 fish may be screened in a day. Two different tags were used during the screening process, numbered Peterson discs were used in 1979 and in 1980 numbered cinch-up Floy tags. While costing approximately two to three times as much as Peterson discs, the cinch-up Floy tags were easier to affix to the fish and were retained longer. The higher cost of the Floy tags was more than offset by their increased efficiency.

A negative aspect of the tagging process was the mortality increase observed during pre-emergent development of the fry. Because no changes were observed in the frequencies of the markers, the 40% mortality in the 1979 brood and 48% mortality in the 1980 brood, can best be attributed to the increased handling of the brood stock, especially the females, required by the

screening process. This increased mortality should be a one-time only phenomenon, but must be taken into account where egg-take goals exist.

For both runs a second generation of marking was performed by using only fin-clipped returns as hatchery brood stock. The rationale for this second effort was that approximately one-half of the production of late run, upstream spawners resulted from natural production of the stream and one-half from the hatchery. Those produced naturally in the first marking generation would not be marked, or worse, be "negatively marked" since many were produced by fish rejected as brood stock for genetic marking in the hatchery. Since the fin-clipped fish were genetically marked, another generation of production should further increase the frequency of the marker allele in the late, upstream spawners. Fin clipping of released genetically marked fry was repeated only in 1982.

Evaluation of the genetic marks

The increase in pre-emergent mortality relative to that normally expected in a hatchery was attributed to the marking process, not the genetic marker itself. To study the effect of the genetic mark, an inferential measurement must be employed. The rationale is that if the marker allele (or alleles closely linked to the marker) is deleterious, individuals possessing the marker are less fit than those not possessing it. As a result of natural selection, the relative frequency of individuals carrying that allele and, therefore, the frequency of the allele will

decrease from generation to generation. There are several different selection regimes that could be considered (Gharrett et al. 1983), but for all genetic marking experiments in which the marker allele is selected against in some manner, a decrease in the frequency of the marker allele can be expected.

The possibility of detrimental effects directly attributable to the genetic marker, or loci closely linked to it, was examined by comparing the frequencies of the marker allele at different times during the salmons' life cycle and between generations. Genetically marked salmon were sampled prior to the release of fry and upon their return as adults, recognizable by fin clips. These allelic frequencies were compared and then pooled for comparison to the frequency determined for their parents, the brood stock for genetically marking these fish.

No significant change in allelic frequency of either genetic marker was observed during the first cycle of either the odd- or even-year experiment. The frequency of the odd-year marker remained at approximately 0.5 (Tables 1a and 1b). The sample available for the returning odd-year genetically marked fish was sufficient to detect decreases in fitness of the whole group of marked fish as small as 7% (Gharrett et al. 1983). While the frequencies observed for the different groups of even-year genetically marked fish were comparable, the returns of late run pink salmon in 1982 (including the genetically marked population) were very low (Table 2a). The non-significance of the frequency comparisons should be considered in view of the fact that the test was only capable of detecting selection that decreased the fitness of the population by 35% (Table 2b).

Table 1a. Allelic frequency data for the odd-year genetically marked pink salmon run in Auke Creek. Relative mobilities of the alleles are in parenthenthes.

Collection	N	MDH-3,4 (70) Frequency	MDH-3,4(130) Frequency
1979 adults screened for brood stock	3906	0.0535	0.0459
1979 brood for genetic marking	390	0.5082	0.0000
1980 fry	658	0.4843	0.0000
1981 genetically marked returns	1048	0.5095	0.0000
1981 brood for genetic marking	412	0.5303	0.0000
1982 fry	649	0.4499	0.0000

Table 1b. Log-likelihood ratio analysis of odd-year genetic marking data.

Source of Heterogeneity	G	degrees of freedom	Probability
Between 1980 fry and 1981 fin-clipped returns	2.054	1	0.15
Between 1979 hatchery brood and pooled offspring (1980 fry and 1981 returns) marking and	0.179	1	0.67
Total	2.233	2	0.33
Between 1981 hatchery brood and 1982 fry	13.056	1	< 0.01

Table 2a. Allelic frequency data for the even-year genetically marked pink salmon run in Auke Creek. The relative mobility of the allele is in parentheses.

Collection	N	AGP (200) Frequency
1980 adults screened for brood stock	7380	0.1988
1980 brood for genetic marking	396	0.8763
1981 fry	695	0.8914
1982 fin-clipped returns	202	0.8515
1982 brood for genetic marking	91	0.8077
1983 fry	383	0.7770
1984 fin-clipped returns	74	0.8446

Table 2b. Log-liklihood ratio analysis of even-year genetic marking data.

Source of Heterogeneity	G	degrees of freedom	Probability
Between 1981 fry and 1982 fin-clipped returns	4.578	1	0.03
Between 1980 hatchery brood and pooled offspring (1981 fry and 1982 returns) marking and	0.197	1	0.66
Total	4.722	2	0.09
Between 1983 fry and 1984 fin-clipped returns	3.603	1	0.06
Between 1982 hatchery brood and pooled offspring (1983 fry and 1984 returns) marking and	0.380	1	0.54
Total	3.984	2	0.14

The second generation of marking the even-year brood produced results similar to the those of the first year (Tables 2a and 2b). Again returns were poor, not just to the hatchery, but throughout the Juneau area. Whether the poor return is attributable to poor ocean-survival or interception by commercial fisheries is unclear, but there is no indication that the genetic marker was responsible. One of the strategies in choosing the AGP locus for marking the even-year brood was to produce a marker with which natural selection would be easily detected. Poor late run returns, however, precluded such efforts.

For the odd-year brood, the second generation of genetic marking produced data more difficult to interpret. The frequency of the marker allele measured in fry sampled before release in spring, 1982 was substantially lower than that of the parents (Tables 1a and 1b). While selection against the genotypes possessing the marker allele is a possible explanation for this observation, there are alternative explanations. Two explanations involve different kinds of sampling error. It is possible that the fry were sampled disproportionately, note the disparity between frequencies of fry and returning adults in the second even-year marking experiment. A second possibility could result from the disproportionate sampling of gametes from the parents, as a result of hatchery practice. This latter explanation was examined (Gharrett and Shirley, in press); and it was concluded that differences in the potencies of males used in hatchery fertilization could inadvertently produce disproportionate contribution of gametes by some males. Although it is not

possible to choose among these explanations and more than one factor may have contributed to the decrease, there are reasonable explanations for the decrease in marker frequency other than selection.

In addition to the studies described above, Lane (1984) also examined allelic frequencies at approximately 20 other electrophoretically detectable loci looking for genetic effects attributable to genetic marking. No substantial changes were observed at these loci, and no apparent linkage disequilibrium was generated.

The genetic marking experiment succeeded in marking one particular population in each of the odd- and even-year Auke Creek pink salmon runs, the late, upstream populations. If the marker alleles are nearly neutral with respect to fitness of those populations, the frequency of the markers should remain constant from generation to generation unless there is considerable immigration into the marked population from unmarked populations. Further evaluation of the genetic marker can be made simply by sampling Auke Creek for several additional generations. Subsequent to the genetic marking, the only pink salmon culture that has been performed has been for experiments for which all progeny are marked. The populations possessing the genetic markers will persist only by natural reproduction. This will provide a means for measuring the fitness of the marker in a "wild" environment.

Interaction of hatchery and "wild" fish

Genetic interaction between the genetically marked hatchery population and other unmarked populations would result in changes in the frequency of marker allele. Straying of hatchery fish into unmarked populations would increase the frequency of the marker allele in those populations while straying of unmarked fish into the marked population would decrease the frequency in the marked population. By monitoring the frequency of the marker allele in populations near the marked population, the extent of straying (gene flow) can be estimated. If the level of gene flow is low or straying is episodic in nature, it may not be apparent in a single generation; however, the net effect should be measurable over several generations because the process is cumulative.

To examine the extent of interaction between the genetically marked population and other nearby populations, the frequencies of the marker alleles were monitored in six spawning populations, early and late run Waydelich Creek spawners, early and late Auke Creek intertidal spawners, early Auke Creek upstream spawners, and Lake Creek spawners (Lake Creek is part of the Auke Lake drainage) (Figure 3). Recall that the genetically marked hatchery population is derived from the late run, upstream spawners in Auke Creek.

Neither the odd- nor even-year experiments indicated that the genetically marked (late run) fish appeared in the early run or strayed into Waydelich Creek (Tables 3 and 4). The temporal precision of homing appears to be nearly perfect, even though

Table 3. Allelic frequencies and examination of heterogeneity of odd-year pink salmon populations near Auke Creek.

	MDH-3,4				HETEROGENEITY		
	N	1.00	1.30	.70	G	df	Probability
AUKE CREEK							
SPAWNERS FOR GENETIC MARKING							
1979	370	0.489	0.000	0.511			
1981	412	0.470	0.000	0.530	0.564	1	0.45
EARLY INTERTIDAL							
1979	90	0.917	0.022	0.061			
1981	100	0.885	0.060	0.055			
1983	33	0.924	0.015	0.061	1.478	2	0.48
EARLY UPSTREAM							
1979	81	0.877	0.086	0.037			
1981	101	0.921	0.030	0.049			
1983	596	0.900	0.057	0.043	5.684	4	0.22
LATE INTERTIDAL							
1979	100	0.860	0.030	0.110			
1981	98	0.898	0.031	0.071			
1983	88	0.909	0.011	0.080	4.318	4	0.36
LATE UPSTREAM							
1979	3888	0.910	0.036	0.054	-----	—	--
after genetic marking							
1981	392	0.727	0.020	0.253			
1983	1010	0.772	0.019	0.208			
TOTAL WITHIN RUNS					11.48	10	0.32
AMONG RUNS WITHIN AUKE CREEK					30.12	6	<<0.01
TOTAL AUKE CREEK					41.6	16	<<0.01
WAYDELICH CREEK							
EARLY							
1979	43	0.802	0.105	0.093			
1981	99	0.887	0.039	0.074			
1983	125	0.912	0.028	0.060	6.857	2	0.03
LATE							
1979	31	0.887	0.032	0.081			
1981	100	0.950	0.010	0.040			
1983	329	0.915	0.043	0.043	3.921	2	0.14
TOTAL WITHIN RUNS					10.778	4	0.03
BETWEEN RUNS WITHIN WAYDELICH					5.166	2	0.08
TOTAL WAYDELICH CREEK					15.944	6	0.01
LAKE CREEK							
1977	106	0.882	0.066	0.052			
1981	54	0.898	0.056	0.037			
1983	77	0.890	0.039	0.071			
TOTAL LAKE CREEK					2.848	4	0.58
TOTAL WITHIN STREAMS					60.392	26	<<0.01
AMONG STREAMS					3.681	4	0.45
TOTAL HETEROGENEITY					64.073	30	<<0.01

Table 4. Allelic frequencies and examination of heterogeneity of populations of even-year pink salmon near Auke Creek.

		AGP			HETEROGENEITY		
	N	1.00	2.00	.65	G	df	Probability
AUKE CREEK							
SPAWNERS FOR GENETIC MARKING							
1980	396	0.124	0.876	0.000			
1982	90	0.194	0.806	0.000	5.633	1	0.02
EARLY INTERTIDAL							
1980	98	0.781	0.219	0.000			
1982	67	0.866	0.134	0.000			
1984	80	0.831	0.169	0.000	4.119	2	0.13
EARLY UPSTREAM							
1978	132	0.784	0.216	0.000			
1980	104	0.793	0.207	0.000			
1982	802	0.796	0.202	0.002	0.835	3	0.84
1984	762	0.784	0.217	0.000			
LATE INTERTIDAL							
1978	64	0.828	0.172	0.000			
1984	80	0.719	0.281	0.000	4.830	1	0.03
LATE UPSTREAM							
1978	244	0.814	0.187	0.000			
1980	7380	0.801	0.199	0.000	0.509	1	0.48
after genetic marking							
1982	635	0.759	0.241	0.000	-----	--	--
1984	528	0.740	0.260	0.000			
TOTAL WITHIN RUNS					10.293	7	0.17
AMONG RUNS WITHIN AUKE CREEK					5.734	3	0.13
TOTAL AUKE CREEK					16.027	10	0.10
WAYDELICH CREEK							
EARLY							
1980	104	0.870	0.130	0.000			
1982	100	0.865	0.135	0.000			
1984	78	0.865	0.135	0.000	0.028	2	0.99
LATE							
1980	102	0.809	0.191	0.000			
1982	100	0.825	0.175	0.000			
1984	28	0.821	0.179	0.000	0.178	2	0.91
TOTAL WITHIN RUNS					0.206	4	>0.99
BETWEEN RUNS WITHIN WAYDELICH					3.951	1	0.05
TOTAL WAYDELICH CREEK					4.157	5	0.53
LAKE CREEK							
1980	99	0.838	0.162	0.000			
1982	76	0.793	0.207	0.000			
1984	47	0.798	0.202	0.000			
TOTAL LAKE CREEK					1.361	2	0.51
TOTAL WITHIN STREAMS					21.545	17	0.20
AMONG STREAMS					9.761	2	0.01
TOTAL HETEROGENEITY					31.306	1919	0.04

genetically marked immigrants would have to comprise at least 5% of the unmarked population to be statistically detectable. Five percent of the early run may be a very small portion of the genetically marked population which generally is much larger than the early spawning populations and the Waydelich Creek populations (Gharrett et al. 1983). For example, a detectable immigration rate of 5% corresponds to 5 genetically marked fish interbreeding with 95 unmarked fish of an unmarked population. Using this immigration rate, if the unmarked population is comprised of 200 individuals, then only 10 marked fish have strayed. If the number of returning marked fish were, say 2000, the extent of straying from the marked population would be only 0.5%, considerably smaller than the immigration rate.

The homing of genetically marked fish to Auke Creek rather than Waydelich Creek also appears strong, although it was not possible to obtain as many samples from Waydelich Creek because the run was often too small. Again the small sizes of the unmarked populations would have enabled us to discover a relatively small number of strays because we were able to sample a large portion of populations.

The picture of spatial homing within Auke Creek provided by the even- and odd-year experiments were quite different. The odd-year marked population appeared to home quite precisely (Table 3). In the small intertidal and Lake Creek populations, even a small level of gene flow from the much larger mainstream population should have been apparent. Spatial precision of homing for the even-year brood, in contrast, definitely indicates gene flow into the intertidal region and suggests the possibility of

straying into Lake Creek (Table 4). Because of the weak AGP mark it was not possible to quantify the extent of straying, but the allelic frequencies suggest that the intertidal and upstream populations are panmictic. Other evidence of straying of the even-year fish was the capture of three fin-clipped fish, presumably from our experiment, at the mouth of Fish Creek in 1982. There is no way to know if these fish would have spawned in Fish Creek.

Failure to observe gene flow from the genetically marked odd-year population does not mean it is nonexistent. It is possible that the level is to low to be resolvable in such a short time or that it is an episodic event. In the latter case it is possible that such an episode occurred during the even-year experiment. Regardless, continued monitoring of this system should clarify the nature of straying.

Because of a portion the Auke Creek production comes from natural reproduction in the creek, the late run fish returning above the weir are a mixture of naturally produced fish and genetically marked fish. Because not all the genetically marked fish were also fin-clipped, an opportunity was presented for applying the genetic marker to a practical problem, estimating the contribution of hatchery fish to the late run. Corollaries to this estimate were estimates of survival of hatchery fish and estimates of mortality attributable to fin clipping.

SUMMARY AND CONCLUSIONS

1) Both even- and odd-year populations of pink salmon in Auke Creek were genetically marked by increasing the frequency of a naturally occurring, but less common, allele through selective breeding.

2) The marking process can increase mortality for that generation as much as 30% as a result of the increased handling.

3) The method used for the marking may be extended to larger scale hatcheries. In such an application, it is recommended that marking be done on two successive generations, the first screening males only, and the second time both males and females.

4) No measurable affect on survival was evident during the first generation of the marking process for either of the marked populations.

5) A decrease in the frequency of the marker allele in the odd-year population may be attributable to spawning methodology.

6) Baseline data showed that the genetic compositions of the populations in the Juneau area are quite similar, suggesting some degree of natural straying among populations.

7) There are large genetic differences in the genetic

compositions of the even- and odd-year runs spawning in the same stream.

8) No straying was observed from the genetically marked late run populations into the unmarked early populations indicating precise temporal homing.

9) No straying was observed from the genetically marked Auke Creek population into the unmarked Waydelich Creek implying accurate homing to stream of origin.

10) The even- and odd-year experiments produced conflicting results concerning the spatial accuracy of within stream homing.

11) The genetic mark was applied to making estimates of hatchery contribution, survival of hatchery produced fish, and fin-clipping mortality.

RECOMMENDATIONS

1) Genetic marking is feasible and is an effective and relatively inexpensive method for uniquely marking a hatchery population. At this time Canadians are using naturally occurring marks in managing their salmon stocks. Considerable application exists for the use of genetic marking in the management of Alaskan stocks.

2) The genetic marking process developed in this study should be extended to a small production facility. Since it is easier to genetically mark a small population than a large one, such a marking effort should be incorporated into the plan for the development of a hatchery, not after the hatchery has been started.

3) The genetically marked systems created by these experiments are capable of producing considerable additional information on the biology and genetics of salmon. An effort should be made to continue collecting data from it.

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