

GENETIC METHOD OF STOCK IDENTIFICATION
IN MIXED POPULATIONS OF SALMON

by

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ABSTRACT

Basic procedures are presented and illustrated for a genetic stock identification (GSI) method that is based on the detection of genetic variability with gel electrophoresis. The method uses naturally occurring genetic differences between stocks to provide estimates of the composition of mixed stock fisheries.

Three examples are given to illustrate the application of the GSI method to management of chinook salmon, Oncorhynchus tshawytscha, fisheries. The examples include: 1) estimates for four potentially contributing populations of fall-run fish intercepted at Bonneville Dam (Columbia River) in 1980 and 1981, 2) an analysis of the 1982 winter gill-net fishery in the lower Columbia River, and 3) an analysis of the ocean troll fishery along the Washington coast during May 1982.

The analytical, economic, and temporal advantages of the GSI method indicate that this procedure is a major new tool for the management of mixed stocks of anadromous salmonids.

INTRODUCTION

A fundamental principle of fishery management is that knowledge of stock composition is required for effective management of mixed stock fisheries (e.g., Larkin, 1981). Absence of this knowledge inevitably results in either overharvest or overescapement. However, such a stock-composition strategy has been practiced only infrequently in the management of anadromous salmonids because of the difficulty in adequately identifying component stock groups. Coded wire tagging (CWT) has given managers a valuable tool for identifying specific groups of hatchery origin, but the method is difficult to use on wild populations and requires considerable effort and cost (Ihssen et al., 1981). Scale analyses have been effectively applied to salmon fisheries (Messinger and Bilton, 1974), but their utility appears to be limited. Also, scale pattern standards can fluctuate between years with changes in environmental conditions, requiring yearly examination and revision of the standards.

An ideal set of stock discriminators should be: 1) expressed independently of environmental changes, 2) comprised of discrete units of information so that population differences can be readily quantified, 3) stable from year to year, and 4) measureable with reasonable efforts and costs. Protein differences detected by gel electrophoresis generally fulfill these requirements. These genetic differences readily accumulate among anadromous salmonid stocks because of the temporal and geographic reproductive isolation associated with the strong homing tendencies of adult salmonids.

The use of genetic data in the management of mixed stock fisheries of anadromous salmonids has been anticipated for over 30 years [for early reviews see Ridgway (1957) and Ridgway and Klontz (1960)]. Early development of the concept came from anthropologists who used the distribution of blood groups to trace patterns of human migration and to identify relationships among major population groups (Mourant, 1954). These studies, coupled with the successful application of blood grouping methods to genetically characterize populations in other mammalian and avian species (Stormont et al., 1951; Briles et al., 1950) suggested that serological procedures might also be extended to characterize breeding units of fish species. However, this idea was abandoned because technical problems limited its application (Hodgins, 1972). Protein gel electrophoresis ultimately provided the quality and quantity of genetic data that had originally been expected from blood groups (Utter et al., 1974); among existing stock identifying procedures, electrophoresis most closely approaches the criteria listed above for distinguishing differences among populations (Utter, 1981).

In addition to a reliable means for obtaining adequate volumes of genetic data, statistical and data processing methods were also needed to obtain estimates of stock contributions of mixed populations. A genetic stock identification method (GSI) has recently been developed and tested that meets these needs (Milner and Teel, 1979^{1/}; Grant et al., 1980; and Milner et al., 1981^{2/}). It is evident from our early work that two conditions must be met for a GSI application. First, each stock that could contribute to a particular fishery must be electrophoretically characterized. Second, sufficient differences among these profiles must be

identified to permit measurement of contributions from each contributing stock.

An extensive data base now exists for chinook salmon, Oncorhynchus tshawytscha, populations ranging from California through northern British Columbia (Milner et al., 1983^{3/}). This data base is centered on populations of the Columbia River whose stocks continue to be major contributors to oceanic fisheries from Alaska southward. Proper management of chinook salmon harvests in this area constitutes a major challenge to regulatory agencies (Van Hying, 1973).

This paper outlines the basic procedures for applying the GSI and describes the use of the chinook salmon data base in the analysis of stock contributions to three chinook salmon fisheries of varying complexity. Its purposes are to illustrate the various steps of this procedure and to demonstrate its unique capabilities through actual management applications. The format is intended to provide a complete overview within the main body of the paper. Readers interested in the underlying principles of genetics, statistics, and data processing that are involved in applying the GSI are referred to Boxes A and B.

METHODS

Use of the GSI method to estimate the composition of a mixed fishery can be divided into four steps:

Step I--Develop Electrophoretic and Laboratory Procedures

Initial laboratory work focuses on developing electrophoretic procedures to detect genetic variation in as many proteins as possible. This phase involves a process of screening a large number of

electrophoretic recipes suitable for use with the species of interest (in this case chinook salmon). Basic laboratory procedures are summarized in Box A.

Step II--Collect Baseline Data

The purpose of this step is to genetically characterize the major stocks which may comprise the mixed fishery. Baseline sampling occurs on individual stocks in freshwater habitats. Tissues from 50 to 100 fish per locale are collected. Genetic data are obtained for the protein systems identified in Step I; the frequencies of the different genetic variants for each protein system are tallied. Data are compared statistically to determine if significant differences exist among collections. Data from collections which are not significantly different are combined, and the resulting individual and combined data are used as the baseline for mixed fishery analysis.

Step III--Collect Mixed Stock Fishery Data

Fish taken from a mixed stock fishery are screened for electrophoretic variability at the same set of protein systems included in Step II. The sample size required for reliable estimates of stock composition is an important but complicated variable to determine. Factors affecting the required sample size include the degree of genetic differences among baseline stocks, the actual or potential number of contributing stocks, and the detail of stock resolution required for management purposes.

Step IV--Estimate Stock Composition

The genetic profiles for the baseline stocks and the mixed fishery samples are used to produce statistical estimates of individual stock or stock group contributions to the fishery. Details of the statistical procedures and the associated validation studies are given in Milner et al. (1981^{2/} and 1983^{3/}).

APPLICATIONS

Three applications of the GSI in analyzing population mixtures of chinook salmon are described in this section (sampling locations are presented in Figure 1). These descriptions, representing successively more complex situations, are provided as examples of diverse management uses of the GSI.

Upper Columbia River Egg Bank

Two morphological types of chinook salmon migrate past Bonneville Dam on the Columbia River during the fall: tules and brights. Tules are dark, mature fish returning to hatcheries in the lower and midportion of the Columbia River; brights are silvery fish, destined mainly for natural spawning grounds in the Hanford Reach area of the Columbia River. Smaller populations of bright fish spawn in the Snake River and in the Deschutes River (Pattillo and McIsaac, 1982). To increase the returns of the upriver bright portion of the Columbia River runs, eggs from fall-run chinook salmon at Bonneville Dam have been selectively used in an "egg-bank" program. GSI analyses of the egg bank interceptions were needed to estimate the relative proportions of the component populations to assure predominance of the Hanford Reach component and to minimize the impact on the drastically depleted Snake River run (Utter et al., 1982^{4/}).

Genetic profiles were obtained for the three components of the bright stock and for tules from the Spring Creek National Fish Hatchery (NFH). Spring Creek NFH is not only the major contributor to the tule run, but, based on the genetic similarity among tule hatchery populations, is also genetically representative of other tule populations. GSI estimates of

stock composition at Bonneville Dam were made for two consecutive years (Table 1). The data indicate a predominance of fish destined for the Hanford Reach area (79 and 86 percent) and much smaller contributions to each of the other three areas (2 to 8 percent). These estimates measure the impact of interceptions on the spawning populations involved and provide a better understanding of the genetic makeup of the resultant egg bank stocks.

Columbia River Winter Gillnet Fishery

A winter gill-net fishery occurs near the mouth of the Columbia River. This fishery is regulated by a variable--and usually brief--opening, because of the limited abundance of returning fish and concerns for adequate escapement to areas above Bonneville Dam to satisfy Indian treaty quotas (Oregon Department of Fish and Wildlife and Washington Department of Fisheries, 1981). A GSI analysis was conducted in cooperation with the Washington Department of Fisheries (WDF) and the Oregon Department of Fish and Wildlife (ODFW) in 1982 on this fishery to determine the proportions of fish returning to a number of areas downstream and upstream of Bonneville Dam.

Genetic estimates were obtained from a data base of 19 potentially contributing stocks and from over 1,000 individuals taken in the gill-net fishery. The estimates for five management units (Table 2) indicate that approximately 90 percent of this fishery was destined for terminal areas downstream from Bonneville Dam with populations from the Willamette River being the predominant contributors. These GSI estimates are similar to estimates obtained from coded wire tagging. For example, CWT information

indicated that 70, 5, and 10 percent of the fishery were destined for the Willamette River drainage, the Kalama River, and the cumulative upriver populations, respectively^{5/}.

The two applications described to this point clearly demonstrate the value of applying the GSI to stock mixtures in the Columbia River. The existing data base is being directly applied to numerous other situations within the river as well, e.g., studying and managing the extensive lower river fishery for fall chinook salmon (chinook salmon are classified as spring-, summer-, and fall-run fish corresponding to discrete seasonal peaks in spawning migrations). It could also be useful in identifying origins of juvenile fish taken at various times and locations in their downstream migration.

Oceanic Fisheries

The most comprehensive application of the GSI described in this article was a cooperative venture with WDF involving the May 1982 troll fishery off the Washington coast (for complete results see Miller et al., 1983, Milner et al., 1983^{3/}). The objectives of this study were to evaluate the GSI as a practical tool for management of oceanic salmon fisheries and to increase the information base for management of this fishery. The analysis included baseline data collected in the region from California through British Columbia, plus approximately 2,000 fish from the troll fishery (Fig. 1) proportionately sampled by area. The results for the overall fishery are presented in Figure 2. The lower Columbia River/Bonneville Pool fall chinook stock group predominated in the fishery over the entire range. Heavy representation of this group in the harvest

is desirable from a management perspective because of the predominance of flourishing stocks of hatchery fish.

As expected, the estimated stock contributions differed between fisheries in the Cape Flattery area in the north and the Columbia River-Grays Harbor area in the south (Fig. 3). For example, there was a higher representation of stocks from British Columbia, Puget Sound, and upper Columbia River fall chinook in the northern fishery. The lower Columbia River/Bonneville Pool group remained the predominant contributor to both areas. However, it represented a significantly lower percentage of the harvest in the northern area. A study of this detail had not previously been possible for an ocean fishery.

Potential for Extended Applications

These three uses of the GSI involving different segments of a common data base and distinct management applications indicate that this procedure has a huge potential for extended application. The method can be applied to any fishery when the following conditions are met: 1) a suitable number of genetic variants have been identified, 2) these variants are distributed among contributing populations at sufficiently different frequencies to permit estimating contributions with reasonable precision at practical sample sizes, and 3) the data base used to estimate contributions is representative of potentially contributing populations. These three conditions appear to have been satisfactorily met in the investigations of chinook salmon outlined in this paper. Migratory salmonids in general tend to meet the second condition; their strong homing instincts favor the formation of genetically discrete populations that can be identified by

electrophoretic techniques. The observed stability of data bases among generations and over year classes (Utter et al., 1980) enhances the value of the GSI. Once adequate baseline data have been collected for a species in a particular area, the primary focus can be on mixed fishery analysis.

The cost advantage of the GSI approach over other procedures used to estimate stock composition is dramatic. The greatest expense in the GSI approach is the collection of the baseline data (i.e., Step II), but once an adequate data base has been collected, the primary focus can be on mixed fishery analysis. Thus, predominant costs for GSI applications are in the collection and analyses of mixed fisheries. A comparison of these costs with those of the CWT approach is necessarily indirect because of the exorbitant expense of tagging wild populations.

Costs for GSI estimates are at least an order of magnitude lower than those of CWT for similar information, even assuming that wild stocks could be tagged and that the cost would be the same as that of hatchery tagging. We have conservatively estimated a total wild and hatchery chinook salmon smolt production from California northward through British Columbia of 251 million (Smith and Wahle, 1981; Perry, 1983^{6/}). Approximately 10 percent of these fish would require tagging to achieve the same levels of precision in estimates as that attained by the GSI method in the 1982 May troll fishery (Milner et al., 1983^{3/}; Osslander, 1983^{7/}). The cost to apply CWT to fish in hatcheries is about \$50/1,000 fish or \$1.26 million for one year class and \$3.78 million for three year classes--the amount required to effectively sample the fishery.

In contrast, contractual costs since 1976 for the development of the chinook salmon baseline data and the procedures for GSI estimation, and for

the collections and analyses of the mixed fishery data (including those described above), total approximately \$650,000. Now that a usable data base has been collected, subsequent costs for similar analyses within this region will be much lower.

The GSI is viewed as a complement to, rather than a replacement for, other procedures for identifying fish in population mixtures (Ihssen et al., 1981). Tagging and marking methods remain necessary for such applications as evaluating experiments and identifying migratory routes of individual fish. Uses of scale characteristics have proven, and will remain, valuable for many applications involving destinations and origins of population mixtures. However, the proven and comprehensive discriminatory powers of the GSI coupled with its potential for in-season management and reasonable costs have opened new horizons for the study and management of mixed stock fisheries. An accelerated use of the GSI therefore appears certain during the current decade as suitable data bases accumulate.

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FOOTNOTES

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Table 1.--Estimates of relative contributions (as percentages) of different stocks of fall-run chinook salmon intercepted for egg bank collections at Bonneville Dam in 1980 and 1981.

| Baseline population | GSI estimates ^{1/} | | Run type |
|---------------------|-----------------------------|------|----------------|
| | 1980 | 1981 | |
| Spring Creek | 7 | 2 | Tule |
| Deschutes | 6 | 5 | Upriver bright |
| Hanford Reach | 79 | 86 | " " |
| Snake River | 8 | 7 | " " |

^{1/} Genetic stock identification estimates based on 1,179 fish in 1980 and 475 fish in 1981, using 9 and 12 variable protein systems, respectively; approximate 95% confidence intervals for the estimates are less than 1%.

Table 2.--Estimated contributions by genetic stock identification method (GSI) of spring-run stock groups to 1982 winter gill-net fishery of chinook salmon in the lower Columbia River.

| Stock group | Estimated contribution (%) (one standard deviation) |
|---------------------------------|--|
| Below Bonneville Dam | |
| Willamette River | 71.7 (0.1) |
| other (Cowlitz-Kalama-Lewis) | 17.2 (0.6) |
| Total | 88.9 (0.1) |
| Above Bonneville Dam | |
| between Bonneville and McNary | 5.8 (0.6) |
| above McNary (Columbia) | 1.8 (0.3) |
| above McNary (Snake) | 3.5 (1.1) |
| Total | 11.1 (1.4) |

Box A. _____ Basic electrophoretic and laboratory procedures.

Insert Box A
Figure here

A. Tissue samples (e.g., muscle, heart, liver, and eye) are taken from each fish and placed in a culture tube with a small amount of water. Cellular proteins in the tissue are released into solution by freeze/thaw and mechanical agitation procedures.

B. A protein extract from each fish is individually absorbed onto a filter paper wick and placed onto the edge of a starch gel at the origin. Samples from 10 fish are shown loaded in the diagram, although typically, samples from 50 fish are loaded on one gel (i.e., with 50 wicks).

C. A direct current is applied across the gel. Protein molecules absorbed on each wick enter and move through the gel because of the molecule's net electrical charge and at a rate proportional to this charge. This charge, in turn, depends on the genetically controlled amino acid substructure of the protein molecules.

D. After about 4 hours, the gel is removed from the power source and the positions of specific proteins (usually enzymes) in the gel are identified by specific histochemical staining procedures (i.e., using general staining reagents or specific procedures involving the enzyme in the staining process). The relative

migration distances of the proteins from the origin, indicated by the staining zones, are recorded as the raw data. The simplified genetic model used for interpreting electrophoretic protein variation is that one gene codes for one protein (polypeptide) chain. Therefore, electrophoretic differences between individuals in protein patterns that are based on amino acid differences are a direct reflection of genetic differences between the individuals. The simple extension of genetic differences between individuals to the evaluation of genetic differences between populations is outlined in Box B.

Box B. The use of electrophoretic data in applying the GSI method.

Insert Box B
Figure 1 here

Data from three gels are illustrated here to demonstrate general electrophoretic results and the classification of genotypes. Each gel contains a sample of 10 fish from one of three populations--A, B, or C. The samples are loaded at the origin and subjected to electrophoresis as outlined in Box A. The position of the enzymatic protein phosphoglucomutase (PGM) is made visible by a histochemical staining procedure specific for PGM. Each of the 10 fish in population A expresses one or both of the mobility forms of the protein PGM: a slow migrating form, S, and a fast migrating form, F. These different electrophoretic expressions are direct reflections of the alleles (alternate forms of a gene) that direct the making of PGM. Fishes 1, 3, and 4 each have a single slow band in Population A. These fish received the same alleles from both parents for the manufacture of the PGM protein and are referred to as SS homozygotes. An SS homozygous individual, therefore, has two doses or copies of the S allele. Fish 8 has a single fast band and is an FF homozygote. Two bands are seen in six individuals of Population A. An individual with a double band has received dissimilar PGM alleles from its parents--here, an S allele from one parent and an F

allele from the other--and is referred to as a SF heterozygote. The combination of alleles, e.g., SS, FF, or SF, that an individual possesses is referred to as its genotype. Genotypic frequencies are simply the proportions of homozygous and heterozygous genotypes for each protein system that are examined.

We have illustrated electrophoretic patterns for a protein that is functional as a single protein chain (i.e., a monomer). Although more complex staining patterns (i.e., phenotypes) can be seen for proteins functional as two or more protein chains, the genetic interpretation for variations of such proteins is parallel to that of monomeric proteins (Allendorf and Utter, 1979); single or multiple banded patterns are expressed by homozygous or heterozygous genotypes, respectively. We have also presented only two alternate alleles for the PGM protein system (S and F). Many protein systems have several allelic forms which increases their contribution to stock discrimination in GSI.

Genotypic frequencies are the fundamental sets of data that are needed to genetically characterize populations and to apply the GSI method. In the figure below, the genotypic proportions of all individuals sampled from a mixed fishery and those of three potentially contributing populations are jointly

examined by a maximum likelihood procedure (outlined in Milner et al., 1983^{3/}) to obtain estimates of the proportion of fish from each potentially contributing stock in the mixture.

Insert Box B
Figure 2 here

Figure 1.—Sampling locations for various seasonal runs of chinook salmon from California through British Columbia. Numbers indicate the 17 stock groups used in the data base for the analysis of the ocean troll fishery:

1. Upper Columbia River, summer-run
2. Lower Columbia River (Cowlitz/Kalama), spring-run
3. Lower Columbia River (Willamette), spring-run
4. Upper Columbia River, spring-run
5. Snake River, spring-run
6. Lower Columbia River/Bonneville Pool, fall-run
7. Upper Columbia River, fall-run
8. California, fall-run
9. California, spring-run
10. Oregon coastal, fall-run
11. Oregon coastal, spring-run
12. Washington coastal, fall-run
13. Washington coastal, spring/summer-run
14. Puget Sound, fall-run
15. Puget Sound, summer-run
16. British Columbia, fall-run
17. Fraser River, summer-run

Figure 2.—Estimated proportions of contributing stock groups to the entire Washington ocean troll fishery, May, 1982. Geographic locations are illustrated in Figure 1 (from Miller et al., 1983^{3/}).

Figure 3.—Estimated proportions of contributing stock groups in northern and southern areas of the Washington ocean troll fishery, May 1982. Geographic locations are illustrated in Figure 1 (from Miller et al., 1983^{3/}).

Box B. (Figure 1) Electrophoretic data and classification of genotypes.

Box B. (Figure 2) Schematic of the GSI method using one variable protein system. In actual application, the power to discriminate between stocks and to estimate their contributions is increased by using the genetic variation found in many protein systems.

Fig. 1

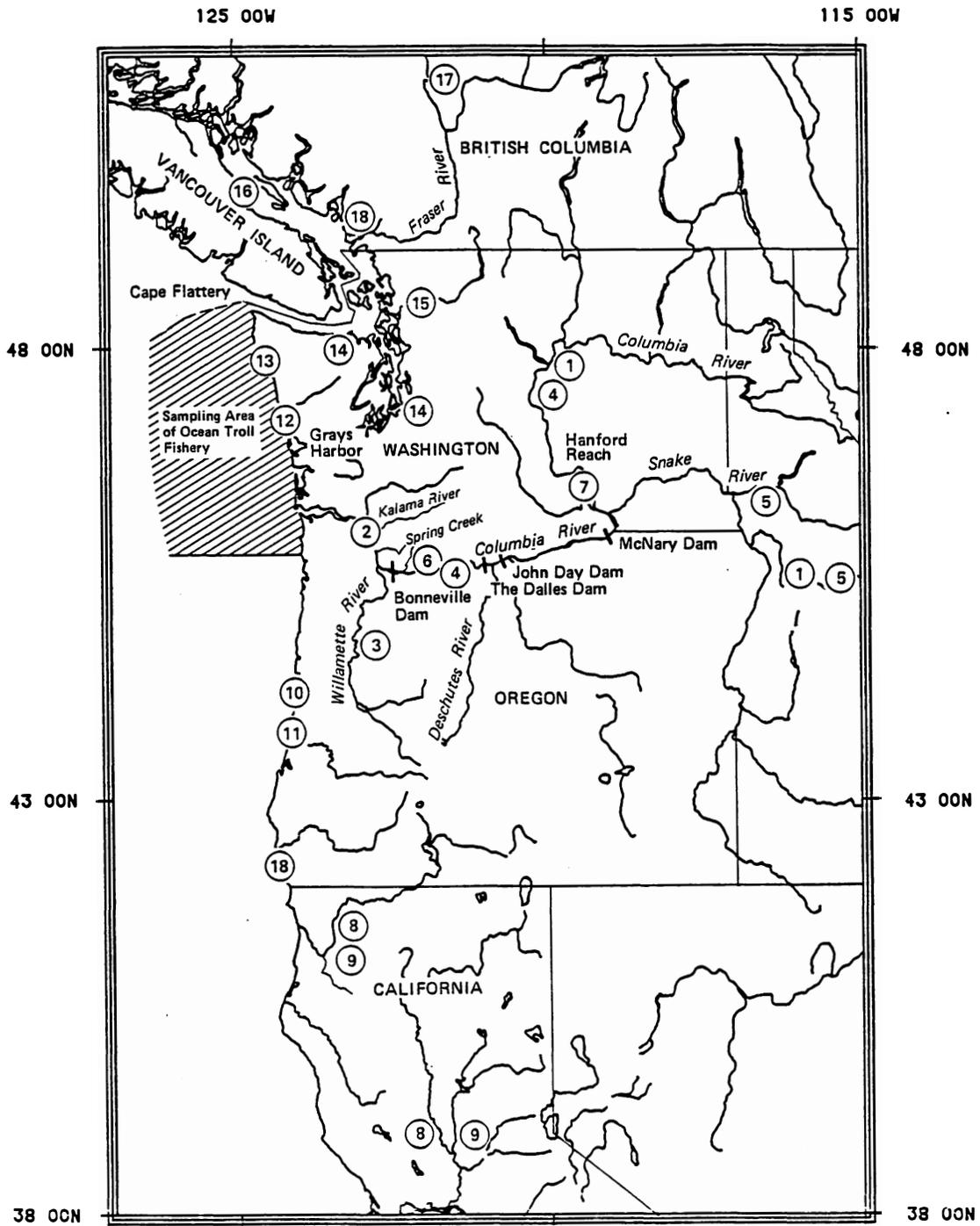


Fig. 2

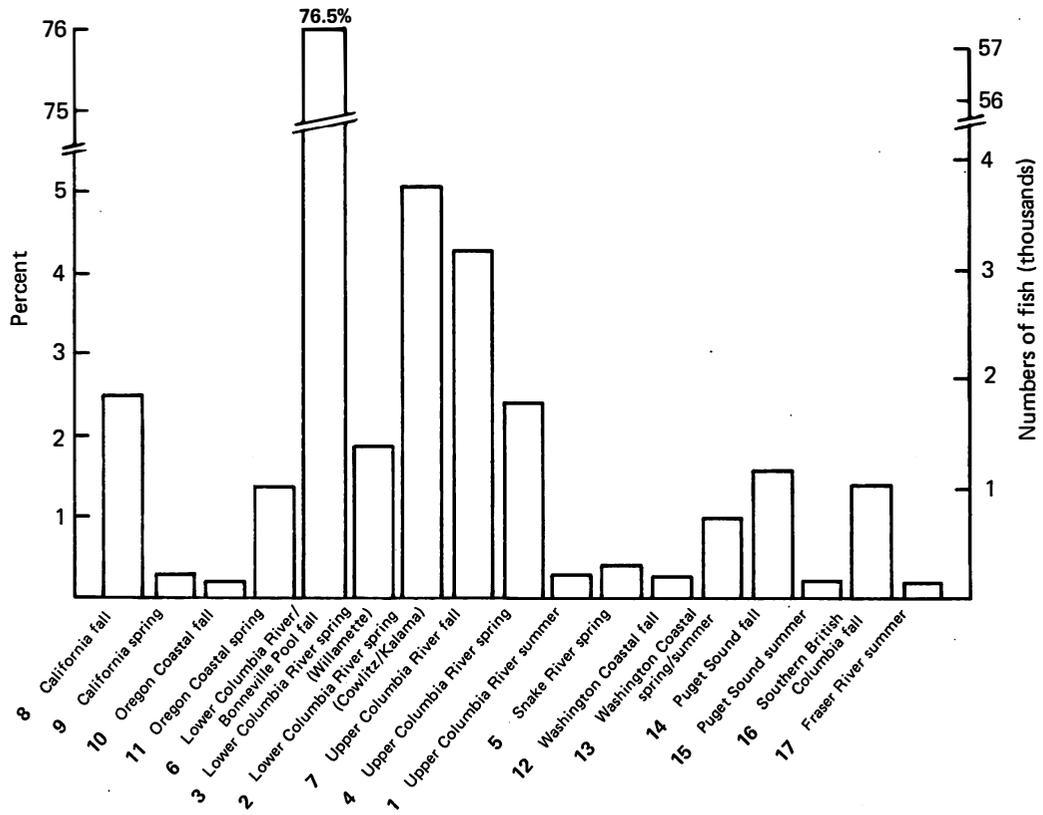
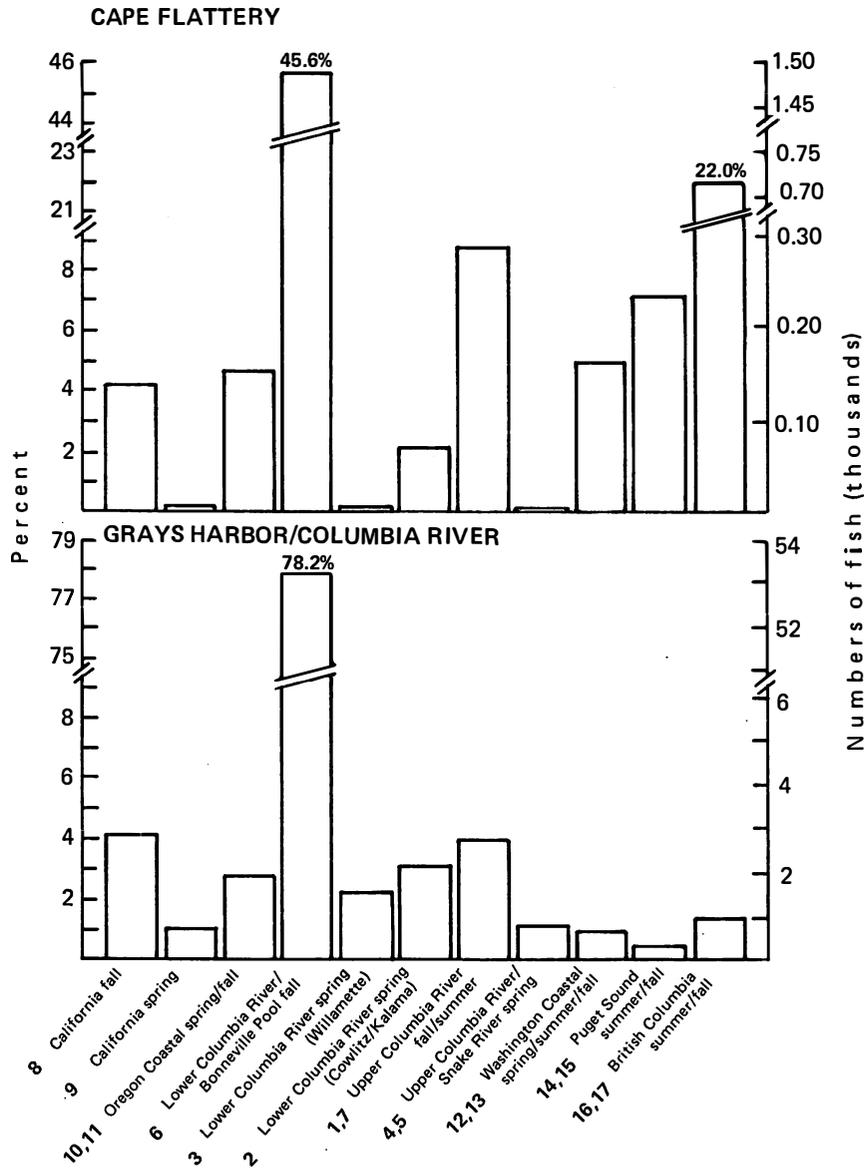
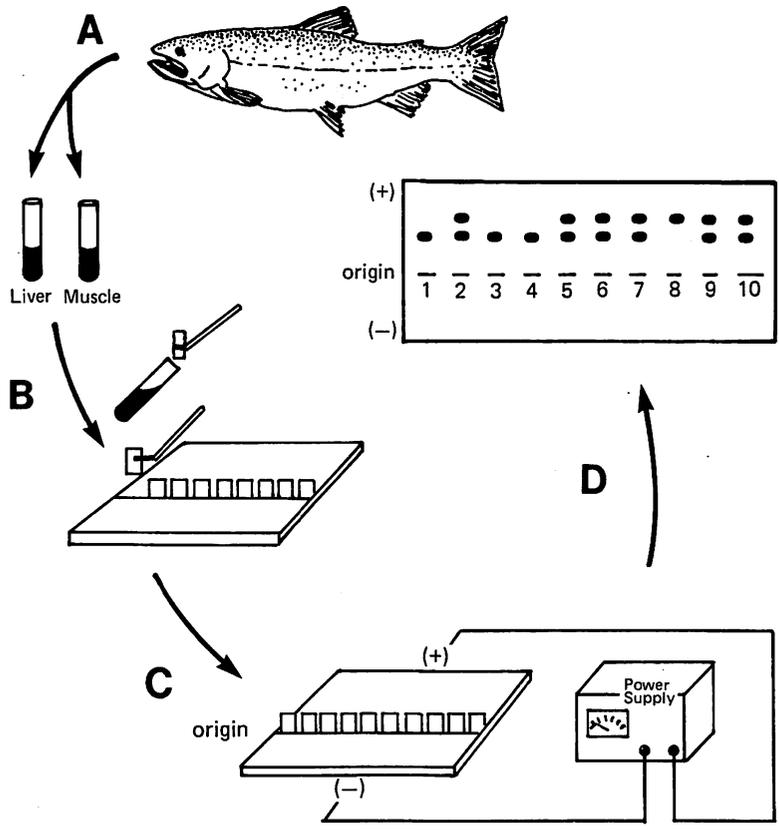


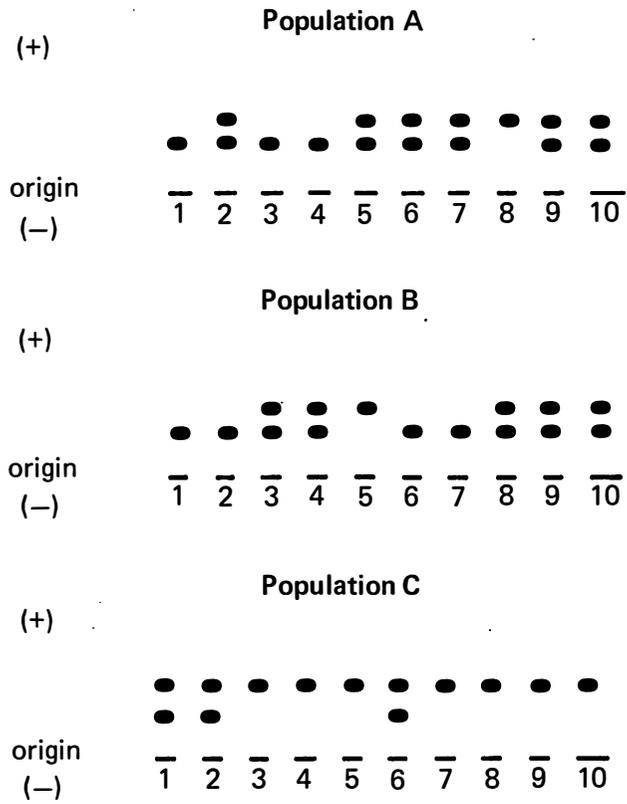
Fig. 3



Box A



Box. B--Fig. 1



| | Genotype frequency | | |
|--------------|--------------------|------|------|
| | SS | SF | FF |
| Population A | 3/10 | 6/10 | 1/10 |
| Population B | 4/10 | 5/10 | 1/10 |
| Population C | 0 | 3/10 | 7/10 |

Box B--Fig. 2

