Salmonid Ecosystems of the North Pacific

Edited by

William J. McNeil
Daniel C. Himsworth

Published by

OREGON STATE UNIVERSITY PRESS
AND
OREGON STATE UNIVERSITY SEA GRANT COLLEGE PROGRAM
Corvallis, Oregon

Library of Congress Cataloging in Publication Data
Main entry under title:

Salmonid ecosystems of the North Pacific.
1. Pacific salmon—Ecology. 2. Fishes—Ecology.
3. Fish-culture—Northwest, Pacific. I. McNeil,
William J. II. Himsworth, Daniel C., 1953-
QL638.S2S24 597'.55 80-14800
ISBN 0-87071-335-3

© 1980 by the Oregon State University Press
Printed in the United States of America
All rights reserved

The Oregon State University Sea Grant College Program is supported cooperatively by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, by the State of Oregon, and by participating local governments and private industry.

This publication was supported in part by the National Marine Fisheries Service.

Population Structures of Indigenous Salmonid Species of the Pacific Northwest

Fred M. Utter, Donald Campton, Stewart Grant, George Milner,
James Seeb, and Lisa Wishard

INTRODUCTION

Studies at the National Marine Fisheries Service Northwest and Alaska Fisheries Center (NWAFC) (Hodgins 1972) to define the genetic structure of salmonid populations had a biochemical basis by the mid-1960s. These early studies (Utter et al. 1974) became the foundation of more recent applications of electrophoretic techniques to evaluate genetic variations of salmonid proteins. Genetic variations defined by electrophoretic studies have been reviewed by Utter et al. (1973), Utter et al. (1974), Utter et al. (1976), and Allendorf and Utter (1979).

The more recent genetic research at NWAFC has centered on populations of *Salmo* and *Oncorhynchus* sp. native to the Pacific Northwest. Although our understanding of the population structures of salmon and trout remains incomplete, our capability to examine specific loci for genetic differences is increasing steadily. This paper summarizes current knowledge on genetic variations in natural populations of sockeye salmon *(Oncorhynchus nerka)*, pink salmon *(O. gorbuscha)*, chum salmon *(O. keta)*, chinook salmon *(O. tshawytscha)*, coho salmon *(O. kisutch)*, rainbow (steelhead) trout *(Salmo gairdneri)* and coastal cutthroat trout *(S. clarki clarki)*.

METHODS

Geographic patterns of genetic variations in protein systems among salmon and trout populations can be used to identify different populations. Protein systems used to evaluate differences among populations are chosen on the basis of their simple inheritance, and genetic differences among populations are evaluated statistically by comparing the frequencies of genotypes or alleles.

Electrophoretic techniques provide an investigator with the capability to collect large amounts of data with relative ease. No other known method is as convenient for making genetic comparisons among congeneric or conspecific populations. Utter et al. (1974) and Allendorf and Utter (1979) describe methods for collecting and interpreting genetic data by electrophoresis. Useful references on histochemical staining include Siciliano and Shaw (1975) and Harris and Hopkinson (1976).

Statistical methods for analyzing data on gene variations among loci, individual fish, populations and species come mostly from procedures developed in studying *Drosophila* and man. The Hardy-Weinberg principle (Stern 1943) is the foundation of many of the analytical processes. It states that the expected genotypic proportions at a polymorphic locus are the square of the allelic frequencies in large, random mating populations where no selective difference exists among genotypes. Significant deviations from expected Hardy-Weinberg proportions may be the result of factors such as drawing the sample from a mixture of populations having different allelic frequencies; a small number of parents giving rise to an inbred population; or selection.

Statistical tests for significant differences between two groups of individuals are made either from genotypic or allelic frequencies. Tests include measuring deviations from expected Hardy-Weinberg proportions, chi-square tests for independence and measuring differences of allelic frequencies from normal approximations of binomial data. Significant differences occurring at a single locus or at several loci can be used to differentiate populations. Summing the differences of allelic frequencies at several loci provides a

basis to calculate the degree of genetic similarity or difference between two populations. Rogers (1972) and Nei (1972) describe two methods for doing this. Calculated values range from 0 (complete genetic difference of loci examined) to 1 (complete genetic identity of loci examined). Dendrograms are sometimes constructed from matrices of such pairwise comparisons among populations (Sneath and Sokal 1973).

Allelic frequencies must remain stable within a population over time to use electrophoretic data to define genetic differences among populations and to estimate contributions of separate populations to mixed fisheries. Estimates taken at different times may be combined to increase the precision of statistical estimators where frequencies remain stable. Unstable frequencies require annual sampling of spawning populations and render the electrophoretic data virtually useless for managing fisheries on mixed stocks.

Allelic frequencies are assumed to remain stable in large populations of salmonids over succeeding generations and among year classes. This assumption is based largely on data where adult and juvenile fish from the same stream tend to express similar allelic frequencies at polymorphic loci, even though the samples include fish from different year classes. Allelic frequency comparisons supporting this assumption are presented in this report and describe different ages, generations and year classes of salmonid populations for which relatively extensive data have been obtained. Enzymes used in these studies are listed in Table 1.

RESULTS AND DISCUSSION

Comparisons of Allelic Frequencies Among Year Classes and Generations

Changes in allelic frequencies of pink salmon have been observed at two locations in Washington. Table 2 presents data for loci (*AGP-1* and *MDH-3*) from five consecutive generations of an early run of pink salmon at the Dungeness River (Aspinwall 1974, Seeb and Grant 1976). These data, along with data for two other loci (*PGM-1* and *AAT-3*) obtained from the Dungeness River and Hoodsport Hatchery populations, provide an opportunity to study changes in allelic frequencies.

There were no observed changes in allelic frequencies for the common alleles of *AGP-1*

Table 1. Protein Enzyme Systems Used in Electrophoretic Comparisons of Genotypes

Enzyme	Abbreviation
Albumin	Alb
Alcohol dehydrogenase	ADH
Alpha glycerophosphate dehydrogenase	AGP
Aspartate amino transferase	AAT
Creatine kinase	Ck
N-acetyl-B-D-Galactosaminidase	GAL
Glutamic-pyruvic transaminase	GPT
Isocitrate dehydrogenase	IDH
Lactate dehydrogenase	LDH
Malate dehydrogenase	MDH
Malic enzyme	ME
Peptidase	PEP
6 phosphogluconate dehydrogenase	6PGD
Phosphoglucose isomerase	PGI
Phosphoglucomutase	PGM
Phosphomannose isomerase	PMI
Sorbitol dehydrogenase	SDH
Tetrazolium oxidase	TO
Transferrin	Tfn
Glycyl leucine peptidase	GL
Leucyl glycylglycine peptidase	LGG
Phenylalanylproline peptidase	PhAP

Table 2. Allelic Frequencies with (in Parentheses) 95 Percent Confidence Intervals Over Sequential Generations for Four Loci in Pink Salmon Collected from Two Areas of Washington.

Location	Year	N	*AGP-1*	*PGM-1*	*AAT-3*	*MDH-3*
Hoodsport	1973[2]	109	.959(.027)	.853(.048)	.719(.061)	.954(.028)
Hoodsport	1975[2]	135	.956(.025)	.828(.046)	.673(.057)	.978(.018)
Dungeness	1969[1]	39	.987(.067)	...	...	.936(.055)
Dungeness	1971[1]	93	.957(.030)	...	...	.962(.028)
Dungeness	1973[2]	35	.943(.055)	.971(.040)	...	.914(.067)
Dungeness	1975[2]	44	.932(.054)	.977(.032)	.549(.106)	.932(.054)
Dungeness	1977	225	.923(.025)	.916(.026)	.708(.043)	.976(.014)

[1] Data from Aspinwall 1974.
[2] Data from Seeb and Grant 1976.

and *MDH-3*. Significant changes occurred both within and between populations at the *PGM-1* and *AAT-3* loci where frequencies in the Dungeness population approached frequencies in the Hoodsport population at both loci. These results were puzzling at first. However, an examination of Washington Department of Fisheries records indicated that fry from the Hoodsport Hatchery had been released from the Dungeness Hatchery in 1976 (1975 brood year). Thus, the run returning to the Dungeness Hatchery in 1977 included survivors from the release of fry originating from Hoodsport plus progeny from natural spawners (Foster et al. 1977). Results are readily explained by: 1) similar frequencies of *AGP-1* and *MDH-3* alleles in the Dungeness and the Hoodsport pink populations, which should remain stable over generations; 2) different frequencies of *PGM-1* and *AAT-3* alleles between the two populations; and 3) an alteration of the *PGM-1* and *AAT-3* frequencies in the 1977 brood year for pink salmon returning to the Dungeness Hatchery as a direct result of large plantings of Hoodsport pink salmon into the Dungeness River.

A second set of data on changes in allelic frequencies pertains to steelhead. Summer-run steelhead from the Skamania Hatchery (Washington State Department of Game) on the Washougal River near Vancouver, Washington have been sampled for five years to study the genetic effect of transplanting stocks. Figure 1 shows that most frequencies of the common allele of a given locus were not significantly different ($P < .05$) between collections. However, the frequencies of the common allele of *MDH-3* from three collections and of the common allele of *TO* from one collection lie outside of the confidence intervals of two or more samples for each of these biochemical systems. These four exceptional frequencies (out of 37 data points) are somewhat higher than the one out of 20 such observations that would be expected from chance alone at the 95 percent confidence level in a stable, random mating population. But the fish returning to the Skamania Hatchery do not represent such a population. The hatchery run was derived from native fish of the Washougal River and also included fish from the Klickitat River approximately 50 miles upstream (personal communication, James Morrow, Washington State Department of Game). Each of the founding year classes comprised a different mixture of progenitor stocks. Some differences among year classes of such a heterogeneous stock would therefore be expected despite factors tending to reduce these differences such as the overlap among year classes and the capability of steelhead for multiple-year spawning. Under these conditions significantly different frequencies between some collections are therefore anticipated. Evidence of allelic frequency stability among cohorts over time is seen in the *MDH-3* frequencies of the juveniles sampled in 1973 and 1974 and in the adults of 1976 and 1977, considering the predominant three-year span from juvenile to adult. These data suggest that the initial inequalities of allelic frequencies expected among year classes may be approaching a point of equilibrium.

Finally, a third set of data exists concerning allelic frequencies from the four loci in juvenile and adult winter-run steelhead trout from the Washington Department of Game Chambers Creek Hatchery (Tacoma, Washington) (Fig. 2). Chambers Creek Hatchery stock was primarily derived from a natural run of Chambers Creek during

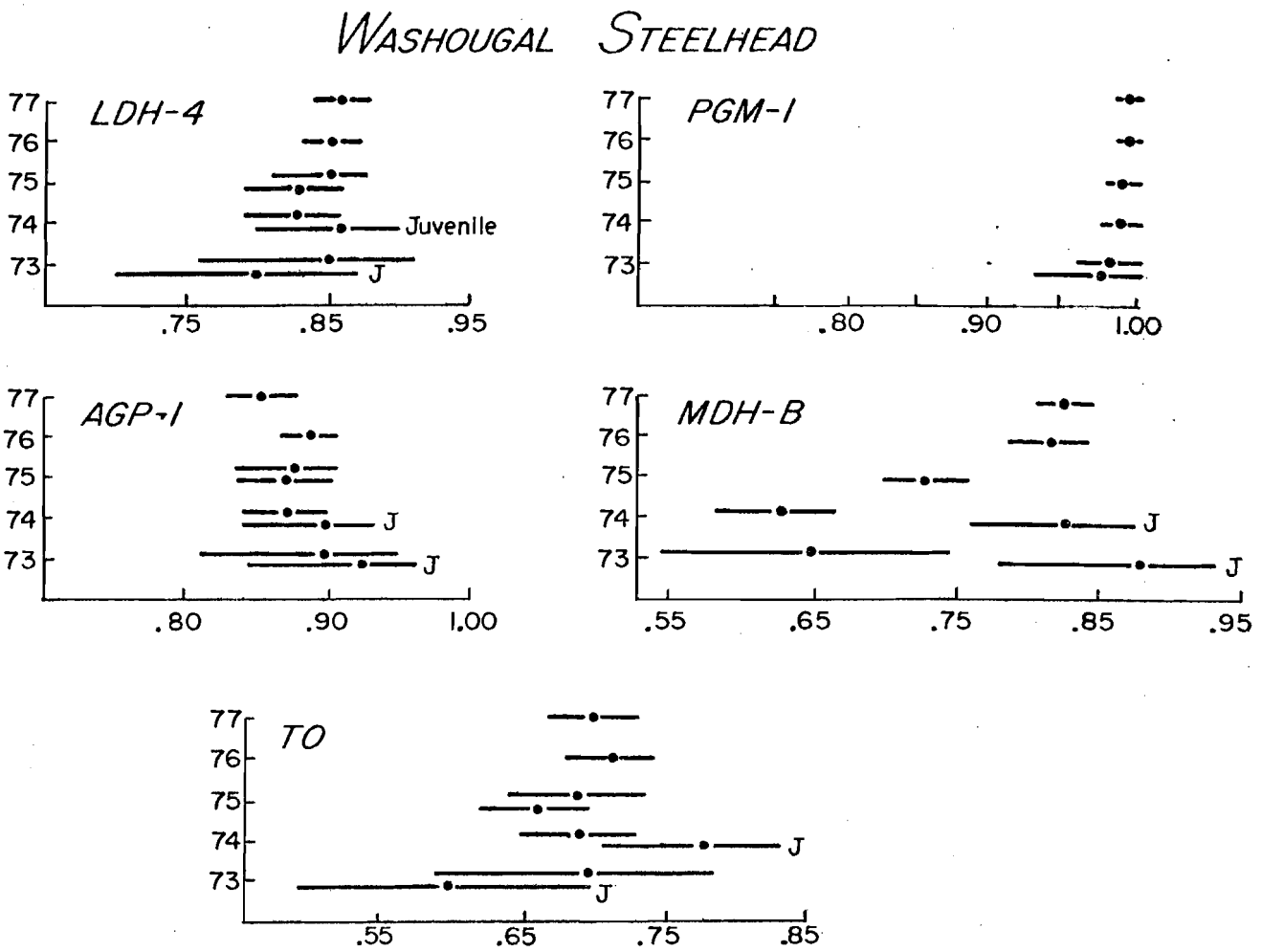

Figure 1. Allelic frequencies and 95 percent confidence intervals at five loci for juvenile and adult Washougal steelhead trout from the Skamania Hatchery over a five year interval.

the late 1940s (personal communication, Art Westrope, Washington State Department of Game). Only a single data point (*MDH-3*) was found to lie outside of the 95 percent confidence intervals. (A single aberrant observation out of 30 lies well within the expected range for this level of confidence). The data indicate that the Chambers Creek steelhead stock presently appears to be close to an equilibrium state with regard to allelic frequencies.

The three sets of data support the assumption of consistencies in allelic frequencies between generations and among (overlapping) year classes. It is important that allelic frequency records over time be maintained, wherever possible, for salmonid populations. Such data are necessary to document the level of genetic consistency within populations, and can provide valuable and unique insights into the causes of changes in populations as they occur.

Genetic Structures of Populations Within Species

This section describes the better known aspects of the genetic variation among salmonids native to western North America. Five salmon species and two trout species are considered.

The number of currently known polymorphic systems in these species (Appendix Table 1) is approximately double the number of variant systems known in rainbow trout five years ago (Utter et al. 1974). This substantial increase in our knowledge of genetic variation, and the likelihood of additional variants being revealed, suggests that a large reservoir of genetic variation exists, which is potentially available for examining genetic structures of salmonids.

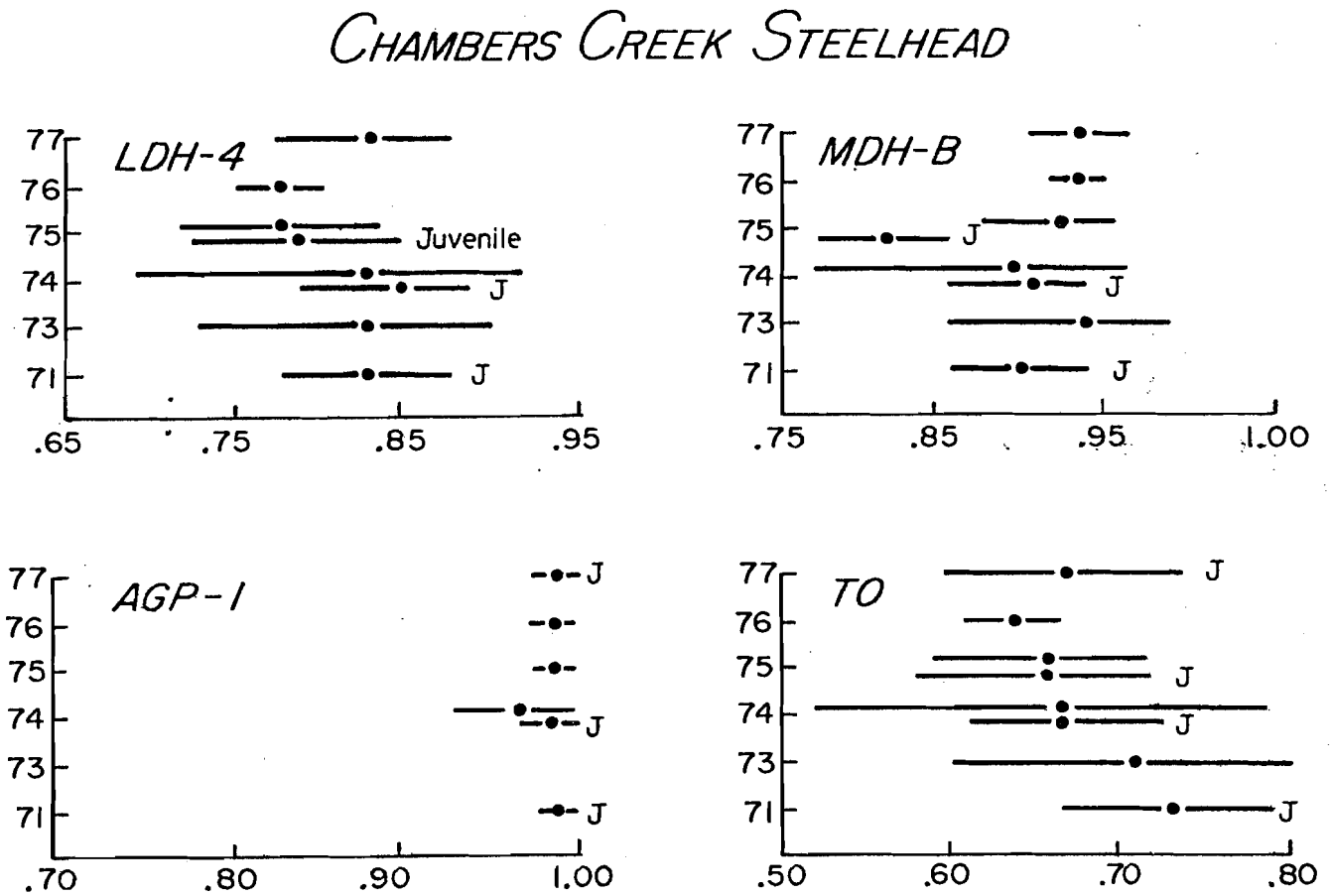

Figure 2. Allelic frequencies and 95 percent confidence intervals at four loci for juvenile and adult steelhead trout from the Chambers Creek Hatchery over a seven year interval.

Sockeye Salmon: Because more precise knowledge was needed about the origins of sockeye salmon harvested in the Japanese high seas fishery, this species was the initial primary target of immunological and electrophoretic studies.

Early electrophoretic data on sockeye were not very useful because alleles of two highly polymorphic systems *(LDH-4, PGM)* occurred at similar frequencies in both American and the Asiatic stocks (Hodgins et al. 1969, Utter and Hodgins 1970, Hodgins and Utter 1971, Altukhov 1975). However, genetic differences among sockeye populations have subsequently been observed to increase southeastwardly from the Copper River drainage in Alaska into regions where stocks do not intermingle significantly with Asiatic stocks. Sampling of sockeye salmon populations from the Skeena River (northern British Columbia) southward to Puget Sound and the Quinault River (Washington coast) indicated higher frequencies of the common *LDH-4* and *PGM* alleles than in populations from western Alaska and Asia (Utter et al. 1974). These differences were also found in non-anadromous populations of sockeye (kokanee). It is now assumed that high frequencies of these two alleles are typical of sockeye populations in the southern part of this species' North American range. Subsequent samplings, however, have proven that this assumption is an oversimplification (Fig. 3). Data from populations sampled southward through the Fraser River drainage into Washington are consistent with the concept of a single major population group (Utter and Hodgins 1970, Hodgins and Utter 1971). However, two collections of anadromous sockeye from Columbia River tributaries (May and Utter 1974) and collections of kokanee from Issaquah Creek near Seattle (personal communication, James Seeb, Washington Department of Fisheries) have allellic frequencies that differ from any other known sockeye population. Fish from these latter locations possess high frequencies of *PGM* variants typically found in populations from western Alaska and Asia. The Issaquah Creek

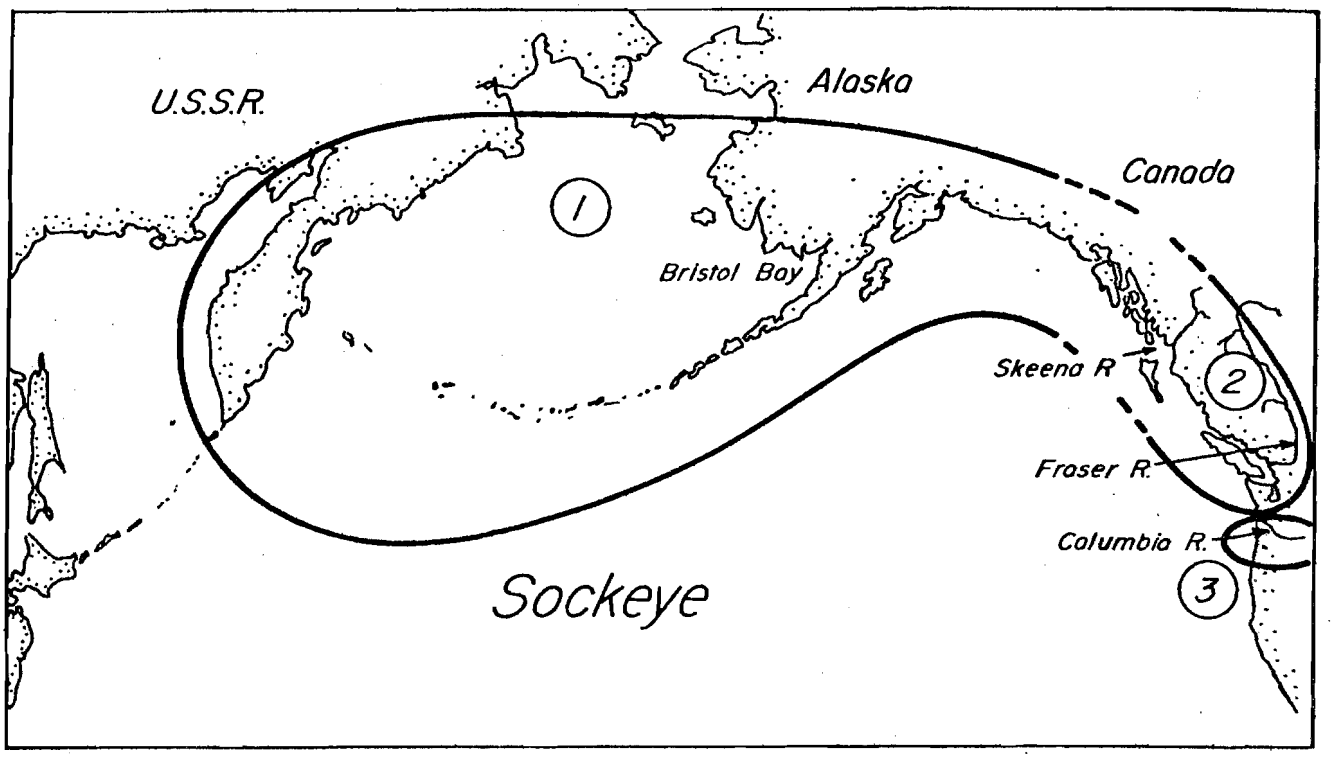

Figure 3. Major population units of sockeye salmon identified through different frequencies of allelic proteins.

kokanee population is also more polymorphic for the same *LDH-4* allele that occurs in the more western populations. Columbia River and Issaquah sockeye also possess variant alleles that have not been observed elsewhere in this species. *MDH-3* and *TO* variants were found in Columbia River sockeye, and a very high frequency of an *LDH-1* variant was found in Issaquah Creek kokanee.

The occurrence of previously undetected alleles in the southeastern extreme of this species' natural range is puzzling. One possible explanation is a diphyletic origin of Washington's sockeye populations following the last glacial era (about 10,000 years ago). One group may have originated from northern populations with the second group descending from southern stocks that may have existed during the last glacial period.

The similarity of allelic frequencies observed within a particular region coupled with low average heterozygosity observed in sockeye when compared to other species (Utter et al. 1973, Allendorf and Utter 1979) caused us to expect little variation in allelic frequencies among different spawning populations within a given drainage. Recent surveys of sockeye populations in the Cook Inlet region of Alaska (Grant et al. *in press*), the Port Alberni region of British Columbia (Allendorf and Mitchell 1977), and the Lake Washington drainage near Seattle (personal communication, James Seeb, Washington Department of Fisheries) have shown considerably greater complexity of allelic frequencies within drainages than expected. Heterogeneity among three drainages supporting sockeye in Cook Inlet was lowest in the river system containing a single lake (Kasilof River) and highest in two river systems containing a complex of lakes (Kenai and Susitna Rivers). The Port Alberni investigations were limited to three lakes in a single drainage where differences in the frequencies of *PGM*, *MDH-3* and *SDH* alleles were sufficient to distinguish fish collected from each of the lakes. The most detailed data were obtained from the Lake Washington drainage (personal communication, James Seeb, Washington Department of Fisheries), which supports both anadromous and non-anadromous populations including Issaquah Creek kokanee. There are large genetic differences among populations of Lake Washington sockeye. Samples from two consecutive years suggest that simulataneous spawning of anadromous and kokanee populations in Issaquah Creek occurs without significant gene flow between groups.

Pink Salmon: Pink salmon populations are characterized by a substantial degree of randomness of allelic frequencies over large geographic areas. Some genetic differences have been observed among odd-year populations in Alaska and the Pacific Northwest, including the Fraser River. An *MDH-B* variant is virtually absent from even-year populations in Alaska, but occurs at varying frequencies in odd-year populations (Aspinwall 1974, Johnson et al. 1978). Frequencies of *AGP* variants appear to be significantly lower in Fraser River and Puget Sound populations than in either even- or odd-year populations returning to Alaska.

For a particular year class, differences observed between even and odd years within a particular stream are generally greater than differences that occur between streams. This peculiar distinction has been recorded by three independent investigations (Aspinwall 1974, Seeb and Wishard 1977*a*, Johnson et al. 1978) and appears to be a direct reflection of the rigid two-year life cycle of pink salmon. Differences contrasting odd-year with even-year fish include generally higher frequencies in odd-year fish for variant alleles of the *AAT-3*, *MDH-3* and *PGM* loci and lower frequencies of *MDH-1* variants.

Attributes of pink salmon populations that distinguish them from other salmonid species include: 1) relatively high amounts of genetic variation within populations (Allendorf and Utter 1979); 2) reasonably large fluctuations of allelic frequencies among breeding groups within limited geographic areas; and 3) minimal patterns of similarity that are useful for defining broad geographic population units for even or odd-year populations. It is possible to interpret these characteristics within the context of the species' life history. Pink salmon spawn largely in the lower reaches and intertidal areas of small coastal streams that are periodically subjected to drastic fluctuations in temperature, and quality and quantity of water. Some streams are also occasionally modified through shifts of spawning strata resulting from earthquakes such as that centered in the Prince William Sound area of Alaska in 1964 (Thorsteinson et al. 1971). Population sizes vary drastically under such diverse conditions (Royce 1962). Allelic frequencies of populations subjected to periodic "bottlenecks" are expected to fluctuate randomly as observed in pink salmon (Crow and Kimura 1970). Thus, the relatively large degree of randomness observed for allelic frequency distributions among pink salmon populations comes as no great surprise. What is surprising are the high levels of heterozygosity in pink salmon populations because periodic "bottlenecks" in the absence of gene influx from other populations are expected to reduce levels of heterozygosity (Crow and Kimura 1970). It is possible that sufficient straying occurs among pink salmon populations to maintain reasonably high levels of heterozygosity, but that this straying is insufficient to overcome the periodic alteration of allelic frequencies brought about by the "bottlenecks."

Chum Salmon: Genetic studies of chum salmon populations were initiated at the same time as sockeye studies because the Japanese fish both species extensively on the high seas in the same areas. Chum salmon have played a subordinate role to sockeye in genetic studies because they are not as important to American fishermen. However, some genetic data on Asiatic chum salmon populations have been published (Numachi et al. 1972, Altukhov 1975).

Three major groups of chum salmon populations are evident from the allelic distribution of two polymorphic loci that both Asiatic and American workers examined. Variants for *LDH-1* are found in virtually all populations examined from southeastern Alaska through Japan (Utter et al. 1973, Altukhov 1975, Seeb and Wishard 1977*b*). *MDH-3* variants are found at polymorphic frequencies (frequencies greater than .01) in Asiatic collections (Numachi et al. 1972, Altukhov 1975, Seeb and Wishard 1977*b*). Neither *LDH-1* nor *MDH-3* variants have been observed from the Fraser River southward despite intensive sampling of Puget Sound populations recently. Thus, three major groups -- tentatively defined as Asiatic, Alaskan and American -- are identified on the basis of variants at two loci (Fig. 4). Discontinuity of sampling precludes any precise definition of boundaries at this time.

Other variants have been useful for examining genetic structures of chum salmon populations in greater detail. Studies by Utter et al. (1973) indicated a lack of genetic variation in chum salmon from Washington based on observations of 20 loci. However, more recent studies have revealed at least six polymorphic loci in Washington populations (*AAT-1*, *2*, and *3*; *IDH*; *PGI*; *GPDP*). There appears to be distinct populations of Puget Sound chum salmon (Seeb and Wishard 1977*b*). Different frequencies of *IDH* alleles tend to separate stocks from north and south Puget Sound, and an important late run of fish entering the Nisqually River is characterized by exceptionally high frequen-

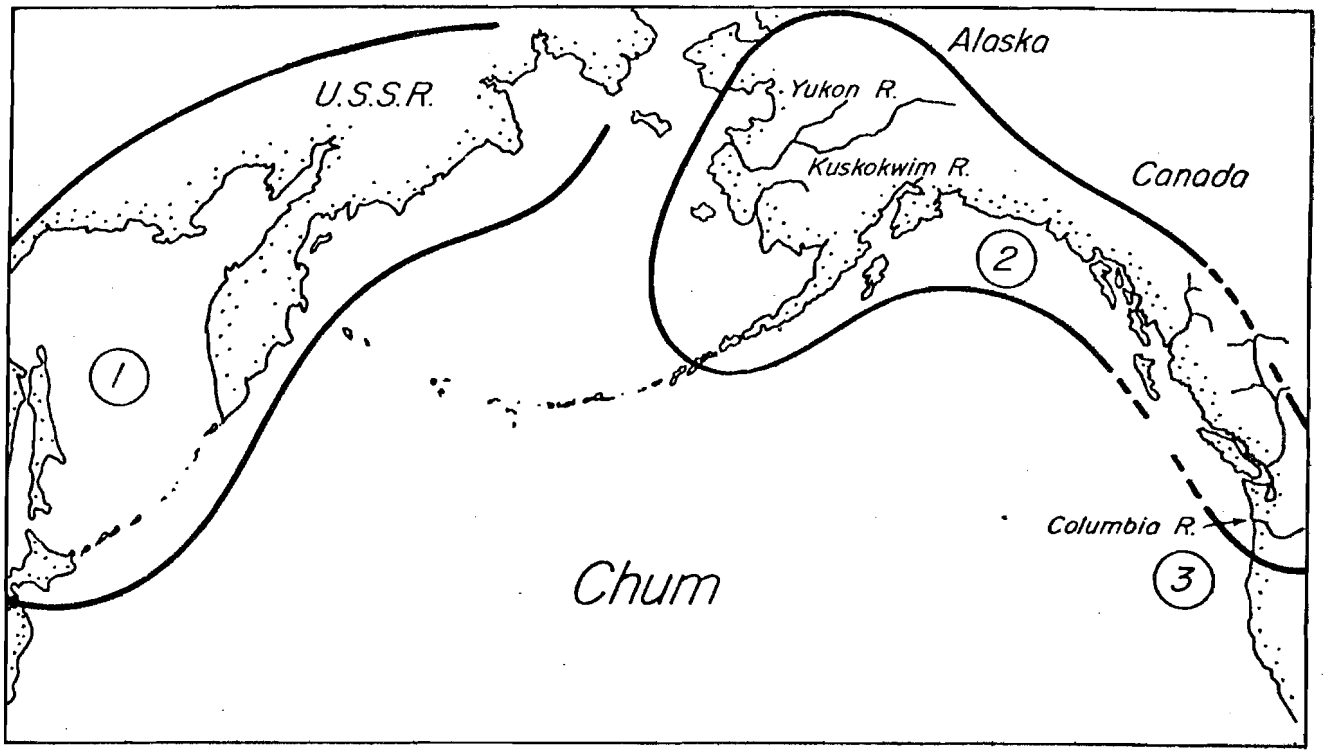

Figure 4. Major population units of chum salmon identified through different frequencies of allelic proteins.

cies of *6PDG* variants. A recent study of chum salmon populations of the Yukon and Kuskokwim drainages (Utter 1978) has indicated an apparent randomness of allelic frequencies within these two drainages except for a slight and consistently different frequency of *PMI* variants between the drainages.

Chinook Salmon: Chinook salmon populations were examined intermittently through collection efforts that focused on sockeye and chum salmon. This work, along with reports from other workers, resulted in a relatively early awareness of two polymorphic systems in chinook salmon, *TO* and *MDH-3* (Bailey et al. 1970, Utter 1971). A stronger emphasis has subsequently been directed toward studying chinook salmon populations, particularly those of the Pacific Northwest. These efforts considerably expanded the number of identified polymorphic systems and a more comprehensive understanding of the genetic structure of chinook salmon populations (May 1975, Kristiansson and MacIntyre 1976, Utter et al. 1976, Milner 1978, Utter 1978).

Chinook salmon populations that extend at least from the Mad River drainage in California through the Quillayute drainage in Washington share similar genetic characteristics. These populations are characterized by uniformly high frequencies of *PGI-2* variation and usually by the presence of *PGM-1* variation (Utter et al. 1976). Only low frequencies of *PGI-2* variants and no *PGM* variants have been observed in the rather extensive sampling of Columbia River and Puget Sound chinook populations. Preliminary data from the east and west coasts of Vancouver Island suggests that these characteristics do not extend into British Columbia populations.

Chinook salmon runs from the Columbia River have been examined for several years. Unlike coastal runs, no clearly identifiable groups of populations have been determined. Fall chinook salmon from hatcheries on the lower Columbia River share the common alleles of the *TO* and *PMI* loci. This may be more a reflection of the indiscriminant exchange of eggs among hatcheries than a close affinity of ancestral stocks (Simon 1972). Spring chinook from the lower Columbia River and its major tributaries tend to be quite distinct from fall chinook returning to this region, principally through considerably higher frequencies of the common *TO* allele.

Chinook populations of the upper Columbia and Snake rivers are tentatively identifiable either through distinctive frequencies of

PMI and *TO* alleles or the presence of low frequency variants at other loci that have not been seen in other populations. These differences must be regarded as provisional because they are generally based on single observations separated by large distances.

A few chinook salmon populations have also been examined in the Yukon and Kuskokwim rivers in Alaska. There appears to be genetic similarity characterized by high frequencies of the common allele of polymorphic loci among the populations from tributaries of both rivers. These populations were dissimilar from all other chinook populations examined except for populations in the upper Snake River drainage.

It is interesting to compare genetic variation within and among chinook salmon populations relative to the overall distribution of the species. The Columbia River is in the center of distribution with major populations both northward and southward. The Yukon and Kuskokwim rivers, on the other hand, are near the northern extremity of the range of chinook salmon in North America (Atkinson et al. 1967). It has long been postulated that populations near the center of the range should have higher levels of genetic variation than populations near the periphery because of the greater potential gene flow (Mayr 1970). Genetic data from chinook salmon support such a hypothesis. The Kuskokwim River and Yukon River populations are generally distinguished from those of the Columbia River by lower amounts of genetic variation among individuals and populations. Only those populations at the inland extremities of the Columbia River drainage (which themselves are peripheral populations within a large river system) resemble the Alaskan populations with regard to reduced levels of genetic variation.

Coho Salmon: The coho salmon has the lowest average heterozygosity value of the five Pacific salmon species in North America (Allendorf and Utter 1979) with only a single highly polymorphic locus - transferrin - among 24 loci examined in a broad survey of populations from California through Alaska. Populations from the Columbia and Fraser rivers can be distinguished from other populations on the basis of a single allele occurring in high frequencies in the former group contrasted with varying, but somewhat equal, frequencies of three alleles in the latter group (Utter et al. 1970, Utter et al. 1973, May and Utter 1974, May 1975, Seidel 1976, Utter et al. 1976, Suzumoto et al. 1977). Experimental data suggest that the distribution of transferrin alleles may be more a reflection of complex bacteriostatic properties of different transferrin alleles than of ancestral relationships (Suzumoto et al. 1977, Pratschner 1978).

Most other loci screened from collections of coho salmon taken throughout the Pacific Northwest are monomorphic. A variant allele of *LDH-4* occurs at moderate frequencies (up to .10) in streams in south Puget Sound and Hood Canal, but is virtually absent in collections from other Pacific Northwest streams (May 1975). Two unusual variants at the *PGM* and *LDH-l* loci occurred at polymorphic frequencies (greater than .05) at the Feather River Hatchery, California (Utter *unpublished data*, May 1975). Both of these variants may occur widely in the southern range of coho. At least some coho populations from the Fraser River and Puget Sound have polymorphic frequencies of variant alleles for *AGPD-3*, *AGPD-4* and two peptidase loci (Seeb *unpublished data*, Utter *unpublished data*). Present data are insufficient to define any geographic patterns for these variants. Coho salmon persist as the species with the lowest incidence of detected polymorphism in spite of these additional polymorphic loci. Much of the developmental work on methods to detect polymorphism has been directed at this species because of its apparent high incidence of monomorphism.

The low levels of genetic variation observed among coho populations can be readily explained if this species had a discontinuous distribution and restricted habitats. These factors would contribute to reduced gene flow among populations as in sockeye salmon, which has a similar low average heterozygosity (Allendorf and Utter 1978). However, coho salmon, more than any of its congeneric species, occupies a continuum of populations in diverse habitats over a broad geographic range (Aro and Shepard 1967). The species has readily adapted to transplantation and hatchery conditions (personal communication, R. Pressey, National Marine Fisheries Service) and is presently more abundant than historically over much of its range due to hatchery propagation. It appears anomalous that such an ubiquitous and adaptable species as the coho should express little generic variation in relation to other salmon species. Two opposing possibilities are suggested: 1) the level of genetic variation currently indicated by protein loci is not a valid reflection of genetic variation over the remainder of the coho salmon genome; and 2) the coho salmon has evolved a genome possessing little genetic variation but a highly adaptable phenotype.

Rainbow Trout: The genetic constitution of rainbow trout has been studied more intensively than any other salmonid species. Considerably greater genetic variation of proteins has been reported for rainbow trout than for Pacific salmon (Utter and Hodgins 1972, Utter et al. 1973). More recent studies of anadromous populations (steelhead) in Washington involving more than 30 loci (Allendorf 1975) have supported this conclusion. Continuing studies of rainbow trout place emphasis on more precise definition of Columbia River populations (Milner 1977, 1979). A more extended geographic survey of the gross population structures of steelhead populations based on data from two polymorphic loci *(LDH-4* and *TO)* has recently been published (Utter and Allendorf 1977).

The current data for frequencies of *LDH-4* and *TO* variants define at least two major geographic units of rainbow trout populations (Fig. 5). A coastal group tentatively extends at least from Kodiak Island southward through the Mad River drainage of northern California. Moderate to low frequencies of *LDH-4* variants and moderate frequencies of *TO* variants typify this group. An inland group, found exclusively in the Fraser and Columbia river drainages east of the Cascade range is identified through very high frequencies of *LDH-4* variants and low frequencies of *TO* variants. This major division presumably reflects two distinct lines dating into the last glacial era (Allendorf 1975). The inland group is postulated to have descended from fish migrating into a large freshwater impoundment resulting from the glacial diversion of the upper drainages of the Columbia and Fraser rivers. The coastal group presumably descended from Asiatic or American stocks that existed outside the glacial mass.

Neither the tendency toward anadromy nor the timing of upstream migration appear to be characteristics of distinct evolutionary lines. Allelic frequencies of both resident and migratory populations of a particular region were invariably similar (e.g. steel-

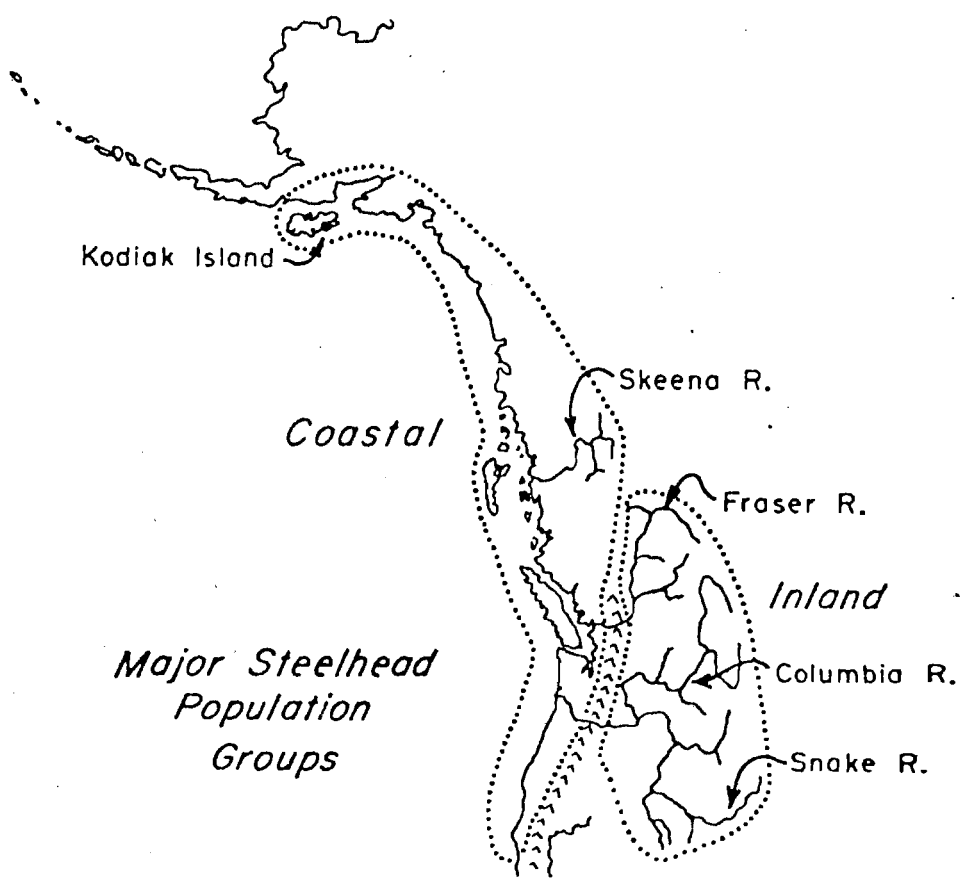

Figure 5. Major geographic units of rainbow trout populations defined by frequencies of *LDH-4* and *TO* variants. (From Utter and Allendorf 1977).

head of the upper Fraser River and "Kamloops" rainbow trout), while summer and winter-run steelhead of a particular drainage tended to resemble one another more than they resembled populations of adjacent drainages (Allendorf 1974; Thorgaard 1977*a*, 1977*b*. Thus, the terms "steelhead," "summer-run," and "winter-run" are useful for the description of life history patterns of a particular population, but are not an indication of close evolutionary relationships among populations of different areas.

The high frequency of *LDH-4* variants in the inland rainbow trout group separates it from the golden trout *(Salmo aquabonita)* and red-band trout. Present evidence indicates that these inland trout groups are closely related to rainbow trout (Gold 1977), but the absence of *LDH-4* variation (Utter and Allendorf 1977) indicates a divergence from the inland rainbow trout populations that predates at least the last period of glaciation.

The boundaries of the coastal group are uncertain because of the absence of sampling at the northern and southern extremes of distribution. The aberrant chromosome counts Thorgaard (1977*b*) observed in steelhead from the Mad River in California indicates a division at this point that was not reflected in the protein data. Non-anadromous hatchery strains Allendorf (1975) examined showed differences between inland and coastal groups. These populations presumably descended from fish taken from the McCloud River drainage of California during the last century (MacCrimmon 1971) and appear to reflect a third major group of rainbow trout populations that may converge with southern populations of the coastal group.

The major population groups of rainbow trout described to this point are virtually certain to have a considerable degree of internal structuring that will become apparent as more populations and loci are examined, and as complementary data are collected by other methods. Such structuring has already been indicated in the coastal group through the cytogenetic studies of Thorgaard (1977*a*, 1977*b*), and the presence of apparently unique protein variants in certain drainages (Allendorf 1975). Direct evidence for a substructure of the inland group was recently found in a comparison of three collections from widely separated areas of the upper Columbia River drainage through differing frequencies of peptidase variants (Milner 1977). The pattern of variation suggests a clear separation of populations from the Columbia River above its confluence with the Snake River and some populations of the Snake River drainage.

Cutthroat Trout: Data have been collected sporadically over a number of years from coastal cutthroat trout, primarily in conjunction with ongoing studies of rainbow trout. Most of these observations were directed toward determining biochemical differences between cutthroat trout and rainbow trout (Utter et al. 1973, Utter et al. 1976, Campton 1980). Cutthroat and rainbow are very closely related, occur sympatrically in many waters and are morphologically indistinguishable in early stages of life history. The ability to electrophoretically identify individuals of the two species and their hybrids has proven to be a valuable asset in stream surveys where both species occur sympatrically, and is routinely used by the Washington State Department of Game (WSDG) for this purpose (Burns et al. 1977). Although we have studied coastal cutthroat trout at the species level for a number of years, virtually nothing was known about the genetic structure of populations of this species prior to 1976 when a formal investigation was initiated to examine this question in conjunction with the establishment of different hatchery lines. These studies have been described by Campton (1980), and have now reached the point where some interesting facts have emerged concerning the population structure of coastal cutthroat trout.

Although cutthroat trout occur from northern California through Prince William Sound, Alaska, the present data are restricted to two proximal regions of western Washington-Hood Canal and northern Puget Sound. These two regions have been examined in considerable detail. Some collections included tributary streams within minor drainages and adjacent areas in a given stream. Coastal cutthroat trout have proven to be the most polymorphic salmonid species studied to date. Over 50 percent of more than 30 loci examined were polymorphic in one or more of the populations examined. Average heterozygosity values in this group of populations are well in excess of .10.

This detailed sampling and testing, coupled with known unique aspects of the life history relative to other indigenous salmonids, has led to new knowledge of this species' biology that has direct implications for its management. A major finding from the protein data was a general unity of populations within each region contrasted

with a difference between regions of a magnitude similar to that separating the coastal and inland groups of rainbow trout. The linear distance separating the two regions is small, but the coastal distance is large because of the hundreds of miles of coastline separating the Stillaguamish River drainage at the southern extreme of the north Puget Sound region and Hood Canal. Tagging studies show that coastal cutthroat trout avoid open, deep water areas such as those separating Hood Canal and north Puget Sound (Johnston and Mercer 1976). This characteristic is consistent with the genetic difference separating populations of the two regions.

The apparent absence of gene flow between Hood Canal and north Puget Sound populations is sufficient evidence to preclude the use of a single hatchery stock derived from either region to enhance fishing in both regions. Interbreeding of native and strongly diverged exogenous fish carries the risk of disrupting highly adapated gene pools, giving rise to suboptimally adapted progeny, and an artificial dependence on continued hatchery supplementation to support the fishery (see Reisenbichler and McIntyre 1976).

One collection of yearling fish from Howe Creek of the Hood Canal region did not conform to the above picture of much greater genetic similarity within a region than between regions. In fact, the collection formed a separate cluster from the remainder of the Hood Canal populations and the cluster of north Puget Sound populations (Campton 1980). This population was also characterized by a low average heterozygosity value relative to all other anadromous coastal cutthroat trout populations examined. These peculiarities were consistent with a hypothesis of one or more population "bottlenecks" affecting the allelic frequencies and levels of genetic variation of this population coupled with an absence of gene flow from adjacent populations. Such a situation would be expected in non-anadromous (resident) populations in a small stream possessing a small population and experiencing little or no outside gene flow into the populations. Campton (1980) postulated that cutthroat residing in Howe Creek represented such an isolated population. This hypothesis was verified in a group of older fish from Howe Creek, that had the same genetic characteristics as young of the year.

A question concerning the systematic status of cutthroat trout native to different areas of the western United States has arisen as a result of cytogenetic and recent biochemical genetic examinations of these fishes (Gold 1977, Campton 1980). *Salmo clarki* presently includes a rather broad assortment of population units that are bound together through morphological similarities but appear to be rather widely diverged on the basis of cytological, biochemical and geographical evidence. Although it is beyond the scope of this paper to review this situation in detail, it is important to emphasize that the coastal cutthroat trout is a distinct taxonomic unit from all other groups of *Salmo* indigenous to western North America, including other groups of *Salmo clarki*. The diploid chromosome number (2N = 68) (Gold 1977) is four higher than any other cutthroat trout group. Coastal cutthroat are restricted to coastal drainages from Alaska through California and are largely sympatric with coastal steelhead trout populations. A comparison of the genetic similarities (measured by about 30 protein loci) among coastal cutthroat trout, "west-slope" cutthroat trout (from Pacific drainages of the Columbia River east of the Cascade Crest), "Yellowstone" cutthroat trout and coastal steelhead trout is particularly interesting. Each of these four groups are about equally diverged from one another, indicating a similar time interval since common ancestry (Campton, 1980). It appears, then, that at least some cutthroat trout lineages have diverged to the species level, and that a thorough multidisciplinary reexamination of the systematics of Pacific species of *Salmo* is appropriate at this time.

GENERAL CONSIDERATIONS REGARDING DATA AND BREEDING STRUCTURE

The present data amplify earlier conclusions from a less extensive survey of three species (coho and chinook salmon and rainbow trout) where a uniqueness of population structuring within a species was noted (Allendorf and Utter 1979). It has become increasingly evident that genetic data derived for one species are insufficient for drawing inferences regarding structures of closely related salmonid species. Differences in genetic structure become more evident from the data presented for seven species in this report, which also discusses possible causes and practical implications of genetic differences. In this section we will discuss four questions related to general aspects of genetic differences.

What are the principal causes of these different patterns of variation in genetic structure? This question lies at the center of one of the fundamental controversies of modern biology, i.e., is such genetic varia-

tion predominantly a reflection of processes of natural selection that directly affect the protein loci examined, or is most of this genetic variation more an echo of random events such as genetic drift or migration? The controversy is presently unresolved, in spite of the intense effort and thought that has been directed toward a solution for more than a decade, mainly because of the difficulties in obtaining reliable estimates of sizes of natural populations (Lewontin 1974). In large, random breeding populations, the effects of natural selection are much more apparent for loci where different coefficients of selection exist for a set of genotypes, while random factors tend to predominate in the distribution of genotypes in small populations (Nei 1975). The limited fecundity and reasonably small population sizes of salmonids places this group of organisms among those where genotypic distributions of natural populations would be principally a reflection of random factors. The above argument does not resolve the issue of selection vs. neutrality of polymorphic protein loci, but does provide a functional framework for interpreting most of the allelic frequencies observed in natural populations of salmonids. Thus, the distribution of protein variants described in this report for seven salmonid species (with the possible exception of transferrin variants of coho salmon) has been interpreted to result predominantly from random processes. This interpretation is supported by the following observations: 1) the major patterns of distribution apparently reflect geographic rather than environmental variables; 2) distinct allelic frequencies are observed in even and odd-year pink salmon from the same stream; 3) consistent allelic frequencies are observed among age groups, year classes and generations in "closed" populations; and 4) successfully transplanted fish obviously influence gene frequencies of the next generation in "open" populations (see Table 2).

It is apparent that allelic frequency data are highly useful indicators of the genetic structures of salmonid species and that the structure of each species is unique. This distinct structuring precludes generalizing from one species to another. Based on protein data collected from different populations of American eels *(Anguilla rostrata)*, Williams et al. (1973) stated "...the recognition of separate Mendelian populations on the basis of significantly different allelic frequencies in a number of other commercially important species is highly questionable..." Our findings invalidate this broad generalization for salmonids and suggest that it may be generally inappropriate.

What are the ultimate limits of population definition based on genetic variations of proteins? This question relates directly to the preceding question. The major factors influencing the frequencies of single gene variants in a population include 1) the number of breeding individuals in a particular generation; 2) the degree of genetic isolation from other populations; 3) the amount of time elapsed since gene exchange occurred from other populations; and 4) the differential influence of natural selection on variants of a particular protein.

We have already explained that the first three factors probably account for most of the variation observed among salmonid populations. Each factor is interrelated and varies from species to species and, to some degree, among populations within species.

Pink salmon populations are a good example of the apparent complexity of those genetic interactions. Pinks may be influenced sufficiently by periodic restrictions of population size within local breeding units to override the potentially stabilizing effects of gene flow between units from possible straying. This combination of genetic dynamics could explain the relatively high amount of genetic variation observed in pink salmon populations and the minimal degree of geographic structuring. The effects of time since gene exchange are apparent through the larger differences seen between year classes than are generally observed within them. The consistency over generations of gene frequencies of Washington pink salmon populations (excepting changes brought about by transplantation) occurred within a period when none of these populations were affected by drastic reductions of breeding individuals. The potential instability of Alaskan pink salmon populations minimizes the value of gene frequency data for estimating ancestral relationships among populations or defining broad geographic population units. However, stable local differences among populations, regardless of cause, are still potentially useful for measuring interactions among these populations (see Table 2).

Anadromous populations of coastal cutthroat trout, in contrast to pink salmon, tend to form distinct population units on a regional basis that appear to reflect ancestral relationships. This contrast is surprising at first glance because of life

history similarities of the two species. Both species spawn in smaller coastal streams and, in a given generation, are potentially affected by drastic reductions in the number of spawning individuals. However, a number of differences exist between the life histories of the two species that appear to influence their genetic structures. Coastal cutthroat trout are capable of breeding more than once and fish of different ages participate in the breeding activities of a given year. Thus, an apparent "bottleneck" for coastal cutthroat trout is not nearly as drastic an event from the point of view of gene frequency stability as it is for pink salmon. A second major difference is that coastal cutthroat trout never venture into deeper saltwater and the potential for gene flow over large distances is therefore much more restricted. Both of these life history differences are consistent with the gene frequency patterns observed in coastal cutthroat trout.

These two examples demonstrate that knowledge of the ultimate resolving powers of gene frequency data in natural populations is dependent upon knowledge of the species' life history in question. This dependency does not reduce the value of gene frequency data for defining population structures. Rather, the dependency works in both directions. As more is known about the genetic structure of a species, more can be deduced about its life history patterns and vice versa.

How much data are required before a pattern of variation among populations of a species becomes evident? Again, there is no single, simple answer to this question any more than to the more general question of the number of points required to resolve trends from graphic projections of any set of data. A logical approach is to initially collect samples over as broad a geographic range as possible and to subsequently focus with more intense sampling on more restricted areas. Single data points are insufficient to define major population groups because of the possibility that differences observed from a particular group of fish may be localized aberrations from the effects of genetic drift. Two collections from proximal populations of a species having similar frequencies at one or more loci that differ significantly from frequencies of these loci in other populations are therefore regarded as the absolute minimum for positive identification of a distinct group of populations.

Will protein data replace traditional methods for studying population structures? The unique properties of genetic variants of proteins (i.e. simple inheritance coupled with ease of detection) permit genetic definitions at the individual, population and species level that were previously impossible to obtain. These capabilities have opened up a new dimension in the study and understanding of natural populations. These expanded capabilities are generally complementary to existing methodologies. External marks and tags remain indispensable tools because of their visibility. Coded wire tags combine relative ease of application and detection with a high potential information content. Intrinsic differences such as those observed in scale characters (Anas and Murai 1969) and trace element composition (Calaprice 1971) are primarily indicators of environmental differences and, as such, are completely complementary to genetic variants of proteins. Genetic variants of proteins are therefore, just one of the valuable tools available for obtaining information about structures and movements of fish populations. It is important that both the capabilities and limitations of a particular tool be properly understood so that the appropriate set of methods can be implemented in a particular study. Nevertheless, genetic data based on protein variation remain singularly superior to any other existing method for defining the genetic structures of populations.

SUMMARY

General consistencies of allelic and phenotypic frequencies at many electrophoretically detected loci have been observed within closed salmonid populations 1) throughout life cycles; 2) over successive generations; and 3) among year classes where overlap occurs. These data support the assumption that patterns of genetic variation observed among populations of these species tend to reflect life history rather than environmental variables. This assumption is the basis for interpreting known patterns of variation in seven salmonid species indigenous to the Pacific Northwest (sockeye, chum, pink, chinook and coho salmon, and rainbow and coastal cutthroat trout). Each species has a characteristic and unique pattern of variation and it is therefore inappropriate to generalize with regard to population structure between even such closely related forms as the two trout species. A major factor affecting the patterns and amounts of genetic variation is the effective population size. This parameter is, in turn, affected by a number

of variables such as discrete or variable year classes, single or multiple spawnings, preferred spawning habitat, degree of anadromy, degree of immigration and emigration, population stability, location of population relative to conspecific populations and time since divergence of two populations. The tendency to form distinct populations in a particular drainage appears to be strongest in the coastal cutthroat and weakest in the pink salmon.

ACKNOWLEDGEMENTS

The outstanding technical assistance of Paul Aebersold and David Teel is gratefully acknowledged. The value of their efforts extended well beyond the collection and analysis of data. They have been jointly responsible for procedures that produce reliable identification of previously undetected polymorphic loci and an overall increase in the efficiency of data collection.

LITERATURE CITED

Allendorf, F. W. 1975. Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. Ph.D. Thesis. Univ. of Wash., Seattle.

Allendorf, F. W. and N. Mitchell. 1977. Genetics of Alberni Inlet sockeye salmon populations. Report to Pacific Biological Station, Nanaimo, British Columbia.

Allendorf, F. W., and F. M. Utter. 1973. Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. Genetics 74:647-654.

Allendorf, F. W., and F. M. Utter. 1976. Gene duplication in the family Salmonidae. III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout *(Salmo clarki)*. Hereditas 82:19-24.

Allendorf, F. W., and F. M. Utter. 1979. Population genetics. Pages 407-454 *in* Hoar, W. S. and D. J. Randall (eds.), Fish physiology. Vol. VII. Academic Press, N.Y.

Allendorf, F. W., F. M. Utter, and B. P. May. 1975. Gene duplication within the family Salmonidae. II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. Pages 415-432 *in* Markert, C. L. (ed.), Isozymes, Volume 4: Genetics and evolution. Academic Press, N.Y.

Altukhov, Y. P. 1975. Population genetics of fishes. Transl. Fish. Mar. Serv. Transl. Ser. No. 3548.

Anas, R. E., and S. Murai. 1969. Use of scale characters and a discriminant function for classifying sockeye salmon *(Oncorhynchus nerka)* by continent of origin. Int. North Pac. Fish Comm. Bull. 26:157-192.

Aro, K. V., and M. P. Shepard. 1967. Pacific salmon in Canada. Int. North Pac. Fish Comm. Bull. 23:225-327.

Aspinwall, N. 1974. Genetic analysis of North American populations of the pink salmon, *Oncorhynchus gorbuscha*, possible evidence for the neutral mutation-random drift hypothesis. Evol. 28:295-305.

Atkinson, C. E., H. J. Rose, and T. O. Duncan. 1967. Pacific salmon in the United States. Int. North Pac. Fish Comm. Bull. 23:43-224.

Bailey, G. S., A. C. Wilson, J. E. Halver, and C. L. Johnson. 1970. Multiple forms of supernatant malate dehydrogenase in salmonid fishes. J. Biol. Chem. 245: 5927-5940.

Burns, D., W. Young, and D. Hanson. 1977. Progress report for the Fish Management Division, Wash. State Dept. Game, Sept. 30.

Calaprice, J. R. 1971. X-ray spectrometric and multivariate analysis of sockeye salmon *(Oncorhynchus nerka)* from different geographic regions. J. Fish. Res. Bd. Can. 28:369-377.

Campton, D. E. 1980. Genetic structure of sea-run cutthroat trout *(Salmo clarki clarki)* populations in the Puget Sound region. M.S. Thesis. Univ. of Wash., Seattle.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper, N.Y.

Engel, W., J. Op't Hof, and U. Wolf. 1970. Geduplikation durch polyploide Evolution: die Isoenzyme der Sorbit-

dehydrogenase bei herings-und lachsartigen Fischen (Isospondyli). Humangenetik 9:157-163.

Foster, B., V. Fletcher, and B. Kiser. 1977. 1976 hatcheries statistical report of production and plantings. Wash. Dep. Fish. Prog. Rep. No. 30.

Gold, J. R. 1977. Systematics of western North American trout *(Salmo)*, with notes on the redband trout of Sheepheaven Creek, California. Can. J. Zoo.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. *In press*. Biochemical genetic variation in sockeye salmon, *(Oncorhynchus nerka)*: tho use of biochemical markers for stock identification in Cook Inlet, Alaska. J. Fish. Res. Bd. Canada.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. American Elsevier, N.Y.

Hodgins, H. O. 1972. Serological and biochemical studies in racial identification of fishes. Pages 199-208 *in* Simon, R. C., and P. A. Larkin (eds.), The stock concept in Pacific salmon, H. R. MacMillan lectures in fisheries. Univ. British Columbia.

Hodgins, H. O., W. E. Ames, and F. M. Utter. 1969. Variants of lactate dehydrogenase isozymes in sera of sockeye salmon *(Oncorhynchus nerka)*. J. Fish. Res. Bd. Can. 26:15-19.

Hodgins, H. O., and F. M. Utter. 1971. Lactate dehydrogenase polymorphism of sockeye salmon *(Oncorhynchus nerka)*. Cons. Int. Explor. Mer., Rapports et proces verbaux; 161:100-101.

Johnson, K. R., R. F. Donelly, W. K. Hershberger, and D. E. Bevan. 1978. Identification of Kodiak Island pink salmon populations based on biochemical genetic variation. Univ. Wash. Coll. Fish. Rep. FRI-UW-7801.

Johnston, J. M., and S. P. Mercer. 1976. Sea-run cutthroat in saltwater pens: Broodstock development and extended juvenile rearing (with a life history compendium). Wash. State Game Dep. Fish. Rep. Project AFS-57-1.

Kristiansson, A. C., and J. D. McIntyre. 1976. Genetic variation in chinook salmon *(Oncorhynchus tshawytscha)* from the Columbia River and three Oregon coastal rivers. Trans. Am. Fish. Soc. 105:620-623.

Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia Univ. Press, N.Y.

MacCrimmon, H. R. 1971. World distribution of rainbow trout *(Salmo gairdneri)*. J. Fish. Res. Bd. Can. 28:663-704.

May, B. 1975. Electrophoretic variation in the genus *Oncorhynchus*: The methodology, genetic basis, and practical applications to fisheries research and management. M.S. Thesis. Univ. of Wash., Seattle.

May, B., and F. M. Utter. 1974. Biochemical genetic variation of the genus *Oncorhynchus* in Pacific Northwest populations: A progress report and program summary. Interim report of contractual work to Wash. Dep. Fish.

May, B., F. M. Utter, and F. W. Allendorf. 1975. Biochemical genetic variation in pink and chum salmon: Inheritance of intraspecies variation and apparent absence of interspecies introgression following massive hybridization of hatchery stocks. J. Hered. 66:227-232.

Mayr, E. 1970. Populations, species and evolution. Belknap Press, Cambridge, Mass.

Milner, G. B. 1977. Biochemical genetic variation: Its use in mixed fishery analysis. Pages 10-15 *in* Hassler, T. J., and R. R. VanKirk (eds.), Genetic implications of steelhead management. Spec. Rep. 77-1.

Milner, G. B. 1978. Final report, Columbia River stock identification study. Submitted to U.S. Fish and Widl. Serv.

Milner, G. B. 1979. Biochemical genetic variation in Columbia River steelhead trout *(Salmo gairdneri* richardson*)* populations and its use in mixed fishery analysis. Ph.D. Thesis. Univ. of Wash., Seattle.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106:283-292.

Nei, M. 1975. Molecular population genetics and evolution. Nort-Holland, Amsterdam.

Numachi, K., Y. Matsumiya, and R. Sato. 1972. Duplicate genetic loci and variant forms of malate dehydrogenase

in chum salmon and rainbow trout. Bull. Jap. Soc. Sci. Fish. 38:699-706.

Perriard, J., A. Scholl, and H. Eppenberger. 1972. Comparative studies on creatine kinase isozymes from skeletal-muscle and stomach of trout. J. Exp. Zoo. 182: 119-126.

Pratschner. G. A. 1977. Relative resistance of six transferrin phenotypes of coho salmon to cytophagosis, furunculosis and vibriosis. M.S. Thesis. Univ. of Wash., Seattle.

Royce, W. F. 1962. Pink salmon fluctuations in Alaska. Pages 15-34 *in* Wilimovsky, N. J. (ed.), H. R. MacMillan lectures in fisheries, Symposium on pink salmon.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Publ. 7213:145-153.

Reinsenbichler, R. R., and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Bd. Can. 34:123-128.

Reinitz, G. L. 1977. Inheritance of muscle and liver types of supernatant NADP-dependent isocitrate dehydrogenase in rainbow trout *(Salmo gairdneri)*. Biochem. Genetics 15:445-454.

Seeb, J., and W. S. Grant. 1976. Biochemical genetic variation in coho, chinook, chum and pink salmon: The use of electrophoretic markers in stock identification. Wash. Dep. Fish. Final rep. service contract 711.

Seeb, J. and L. N. Wishard. 1977*a*. Genetic characterization of Prince William Sound pink salmon populations. Report to Alaska Dep. Fish Game.

Seeb, J., and L. N. Wishard. 1977*b*. The use of biochemical genetics in the management of Pacific salmon stocks: Genetic marking and mixed fishery analysis. Wash. Dep. Fish. Final rep. service contract 792.

Seidel, P. R. 1977. Early life history study of three Washington Department of Fisheries coho salmon *(Oncorhynchus kisutch)* populations by interracial hybridization. M.S. Thesis. Univ. of Wash., Seattle.

Siciliano, M. J., and C. R. Shaw. 1976. Separation and visualization of enzymes on gels. Pages 185-209 *in* Smith, I. (ed.), Chromatographic and electrophoretic techniques, Vol. 2, Zone electrophoresis. Heinemann, London.

Simon, R. C. 1972. Gene frequency and the stock problem. Pages 161-196 *in* Simon, R. C., and P. A. Larkin (eds.), The stock concept in Pacific salmon. Inst. Fish. Univ. British Columbia, Vancouver.

Sneath, P. H., and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco.

Stern, C. 1943. The Hardy-Weinberg law. Sci. 97:137-138.

Suzumoto, B. K., C. B. Schreck, and J. D. McIntyre. 1977. Relative resistances of three transferrin genotypes of coho salmon *(Oncorhynchus kisutch)* and their gematological responses to bacterial kidney disease. J. Fish. Res. Bd. Can. 34:1-8.

Thorgaard, G. H. 1977*a*. Chromosome studies of steelhead. Pages 20-25 *in* Hassler, T. J., and R. R. VanKirk (eds.), Genetic implications of steelhead management. Calif. Coop. Fish. Res. Unit. Spec. Rep. 77-1.

Thorgaard, G. H. 1977*b*. Chromosome rearrangements and sex chromosomes in the rainbow trout and sockeye salmon. Ph.D. Thesis. Univ. of Wash., Seattle.

Thorsteinson, F. V., J. H. Helle, and D. G. Birkholz. 1971. Salmon survival in intertidal zones of the Prince William Sound streams in uplifted and subsided areas. Pages 194-219 *in* The great Alaska earthquake of 1964: Biology. Nat. Acad. Sci. SIBN 0-309-01604-5.

Utter, F. M. 1971. Tetrazolium oxidase phenotypes of rainbow trout *(Salmo gairdneri)* and Pacific salmon *(Oncorhynchus* sp.) Comp. Biochem. Physiol. 39B:891-895.

Utter, F. M. 1978. Genetic variants of proteins in chum and chinook salmon from the Bering Sea and the Yukon and Kuskokwim rivers: Report of analyses of 1976 collections. Rep. to R.E.F.M. Div., Northwest and Alaska Fish. Center and the Alaska Dep. Fish and Game.

Utter, F. M., and F. W. Allendorf. 1977. Determination of the breeding structure of steelhead populations through gene frequency analysis. Pages 10-15 *in* Hassler, T. J. and R. R. VanKirk (eds.), Genetic implications of steelhead management. Calif. Coop. Fish. Res. Unit. Spec. Rep. 77-1.

Utter, F. M., F. W. Allendorf, and H. O. Hodgins. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst. Zoo. 22:257-270.

Utter, F. M., F. W. Allendorf, and B. May. 1976. The use of protein variation in the management of salmonid populations. Pages 373-384 *in* Taber, R. C., and F. Bunnell (eds.), Proceedings of the 41st North American Wildlife Conference.

Utter, F. M., F. W. Allendorf, and B. May. *In preparation.* The genetic basis of creatine kinase isozymes in skeletal muscle of salmonid fishes.

Utter, F. M., W. E. Ames, and H. O. Hodgins. 1970. Transferrin polymorphism in coho salmon *(Oncorhynchus kisutch).* J. Fish. Res. Bd. Can. 27:2371-2373.

Utter, F. M., and H. O. Hodgins. 1970. Phosphoglucomutase polymorphism in sockeye salmon. Comp. Biochem. Physiol. 36:195-199.

Utter, F. M., and H. O. Hodgins. 1972. Biochemical genetic variation at six loci in four stocks of rainbow trout. Trans. Am. Fish Soc. 101:494-502.

Utter, F. M., H. O. Hodgins, and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: Potentialities and limitations. Pages 213-237 *in* Biochemical and biophysical perspectives in marine biology, Vol. 1. Academic Press, N.Y.

Utter, F. M., H. O. Hodgins, F. W. Allendorf, A. G. Johnson, and J. L. Mighell. 1973. Biochemical variants in Pacific salmon and rainbow trout: Their inheritance and application in population studies. Pages 329-339 *in* Schroder, J. H. (ed.), Genetics and mutagenesis of fish. Sprinter-Verlag, Berlin.

Utter, F. M., H. O. Hodgins, and A. G. Johnson. 1972. Biochemical of genetic differences among species and stocks of fish. Pages 98-101 *in* Annual Report for 1970. Int. North Pac. Fish. Comm.

Williams, G. C., R. K. Koehn, and J. B. Mitton. 1973. Genetic differentiation without isolation in the American eel, *Anguilla rostrata.* Evol. 27:192-204.

Wright, J. E., J. R. Heckman, and L. M. Atherton. 1975. Genetic and developmental analyses of LDH isozymes in trout. Pages 375-401 *in* Isozymes, Vol. III. Academic Press, N.Y.

APPENDIX

Symbols Used in Appendix Table 1

A -no variants seen
B -infrequent variants seen
C -polymorphic above one percent allelic frequency
.... -no data available
E -eye
G -gut
H -heart
L -liver
M -skeletal muscle (*MDH-M* is presumed to be mitochondrial locus in skeletal muscle)
S -serum

Abbreviated protein systems include:

Alb -albumin
ADH -alcohol dehydrogenase
AGP -alpha glycerophosphate dehydrogenase
AAT -aspartate amino transferase
CK -creatine kinase
GAL -M-acetyl-B-D-Galactosaminidase
GPT -glutamic-pyruvic transaminase
IDH -isocitrate dehydrogenase
LDH -lactate dehydrogenase
MDH -malate dehydrogenase
ME -malic enzyme
PEP -peptidase*
6PGD -6 phosphogluconate dehydrogenase
PGI -phosphoglucose isomerase
PGM -phosphoglucomutase
PMI -phosphomannose isomerase
SDH -sorbitol dehydrogenase
TO -tetrasolium oxidase (also commonly called superoxide dismutase-*SOD*)
Tfn -transferrin

*different peptidase for *PEP* include glycyl leucine (*GL*), leucyl glycyiglycine (*LGG*), and phenylalanylproline (*PhAP*). This summary excludes esterase and hemoglobin variants, which we do not regularly examine because of problems involving repeatability and instability.

Appendix Table 1. Summary of Variant Protein Systems in Seven Salmonid Species.

Locus	Tissue Distribution	Sockeye	Pink	Chum	Coho	Chinook	Rainbow	Cutthroat	Reference Sources
		Species							
Alb	S,E	...	...	C	A	...	C	...	Altukhov 1975, *unpublished data* of G. A. E. Gall and B. Bentley.
ADH	L	A	A	A	B	C	C	C	Allendorf et al. 1976, Milne 1978, Campton 1980.
AGP-1,2	M	B	C	B	B	B	C	B	Seeb and Wishard 1977*b*, Utter and Hodgins 1972, Aspinwall 1974, May et al. 1975, Utter 1978, *unpublished data* of Seeb, Aebersold and Wishard.
-3,4	M,H	...	C	C	C	...	C	...	
AAT-1,2	M,H	A	A	C	B	C	C	c	May 1975, Allendorf and Utter 1976, May et al. 1975, Allendorf 1975, Seeb and Wishard 1977*a*.
-3	E	A	C	C	A	C	A	A	
CK-A1,2	M	A	A	A	B	A	C	A	Utter et al. *in preparation*, Perriard 1972.
-C1,2	G	...	...	...	...	...	B	...	
GAL	L	...	...	...	C	...	...	...	Aebersold and Wishard *unpublished data*.
GPT-2	M	C	A	A	A	A	A	C	Grant et al. *in preparation*, Campton 1980.
IDH-1,2	L	A	C	C	A	C	C	C	Allendorf and Utter 1973, Seeb and Wishard 1977*a*, Campton 1980, May 1975, Milner 1978, Reinitz 1977.
-3	M	A	A	A	A	A	C	A	
LDH-1	M	A	C	C	C	A	A	A	Utter and Hodgins 1972, Utter et al. 1973, Wright et al. 1975, May 1975, Campton 1980, Johnson et al. 1978, Milner 1978, Seeb and Wishard 1977*b*.
-3	E,M	A	A	...	B	A	A	A	
-4	L,E,M	C	B	C	C	B	C	B	
-5	E	B	A	A	A	C	C	C	
MDH-1,2	L,E,M	A	C	A	A	B	C	C	Bailey et al. 1970, Utter and Hodgins 1972, Numachi et al. 1972, Aspinwall 1974, Altukhov 1975, May 1975, Seeb and Wishard 1977*a*, Campton 1980, Milner 1978, Teel and Milner *unpublished data*.
-3,4	M	B	C	C	B	C	C	C	
-M	M	...	...	...	...	C	...	...	

Appendix Table 1. Summary of Variant Protein Systems in Seven Salmonid Species-continued.

Locus	Tissue Distribution	Sockeye	Pink	Chum	Coho	Chinook	Rainbow	Cutthroat	Reference Sources
		Species							
ME-1	M	A	C	...	A	A	A	A	Seeb and Wishard 1977*a*, Johnson et al. 1978, Campton 1980, Milner 1978, Gall and Bentley *unpublished data*.
-2	M	A	A	...	A	C	C	B	
-3	L	...	...	...	...	...	C	...	
PEP-1(GL)	M,L,E	A	A	A	C	C	C	C	Milner 1977, Campton 1980, *unpublished data* of all authors of this paper.
-2(GL)	E	A	A	A	C	A	A	A	
-3(LGG)	M,L,E	A	A	C	B	C	...	...	
-4(PhAP)	M,L.E	...	...	...	B	...	...	...	
6PGD	M,L,E	A	C	C	A	A	A	C	May 1975, Seeb and Wishard 1977*a*, 1977*b*; Campton 1980.
PGI-1,2	M	A	C	A	B	C	B	C	May 1975, Allendorf 1975, Utter et al. 1976, Seeb and Wishard 1977*a*, Campton 1980.
-3	M,E,L	A	A	A	B	A	C	B	
PGM-1	M	C	C	A	B	C	C	C	Utter and Hodgins 1970, 1972; May 1975, Seeb and Grant 1976, Kristiansson and McIntyre 1976, Campton 1980, *unpublished data* of all authors of this paper.
-2	L,E	C	A	...	B	...	C	C	
PMI	M,L,E	A	C	C	B	C	B	C	May 1975, Seeb and Grant 1976, Milner 1978, Campton 1980.
SDH-1,2	L	A	A	A	B	C	B	C	Engel et al. 1970.
TO-1	L,M	B	A	A	A	C	C	C	Utter 1971, May and Utter 1974, Campton 1980.
-2	H	...	...	...	...	C	...	...	Utter 1978.
Tfn	S,E	A	A	A	C	A	C	C	Utter et al. 1970, Utter and Hodgins 1972, Campton 1980.