

Natural Hybridization between
Coastal Cutthroat Trout (Oncorhynchus clarki)
and Steelhead Trout (Oncorhynchus mykiss)
within Redwood Creek, California.

by

W. George Neillands

A Thesis
Presented to
The Faculty of Humboldt State University

In Partial Fulfillment
of the Requirements for the degree
Master of Science

November, 1990

ABSTRACT

Natural hybridization between Oncorhynchus clarki and Oncorhynchus mykiss residing in Redwood Creek, California was investigated using biochemical genetic data. Trout samples were collected in each of nine Prairie Creek tributaries, five Redwood Creek tributaries, and in the anadromous populations in the Redwood Creek embayment. Horizontal starch gel electrophoresis was performed on liver, eye, heart, and muscle tissues.

Two analytical approaches were used to determine if Oncorhynchus mykiss and Oncorhynchus clarki are hybridizing in Redwood Creek drainage. One method is the hybrid index function which estimates the probability of pure species parentage (Campton and Utter 1985). A second method evaluates the breeding structure of the populations sampled as a deviation from a theoretical, single randomly mating population.

Initial evaluation revealed single interbreeding populations of steelhead trout in Little Lost Man Creek and in Lost Man Creek. A single population of cutthroat trout was identified in Forty-Four Creek, and a single population of trout from mixed ancestry was identified in Tom McDonald Creek. The other populations appear to reflect varying degrees of both trout species.

Upon further statistical evaluation, Bond Creek, Elam Creek, and Redwood Creek embayment populations of trout

appear to be comprised of non-interbreeding populations of cutthroat trout and steelhead trout. May Creek's trout population appears to be a single population of cutthroat trout that have been introgressed by steelhead trout in the recent past. MacArthur Creek, Larry Dam Creek, Wigeon Creek, Boyes Creek, Prairie Creek, Godwood Creek, and Wolf Creek populations appear to be comprised of two distinct trout populations, plus evidence of recent hybridization or introgression into one of the two trout populations.

The existence of natural hybrids between steelhead trout and coastal cutthroat trout in headwater trout populations and their absence from the anadromous coastal cutthroat trout population in Redwood Creek embayment leads to the hypothesis that natural hybridization between these two species is more common in the resident coastal cutthroat trout than in sea-run coastal cutthroat trout.

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
LIST OF TABLES	viii
LIST OF FIGURES.	ix
INTRODUCTION	1
METHODS	9
Electrophoresis	9
Data Analysis.	10
RESULTS	17
DISCUSSION and CONCLUSIONS	32
REFERENCES CITED	39
APPENDIXES	
A Allele Frequencies from Redwood Creek Tributaries .	46
B Allele Frequencies from Little Lost Man Creek and Lost Man Creek	47
C Allele Frequencies from Prairie Creek Tributaries	48
D Allele Frequencies from Redwood Creek Embayment . .	49
E Estimates of Gametic Disequilibrium Among Diagnostic loci for Redwood Creek Tributaries . .	50
F Estimates of Gametic Disequilibrium Among Diagnostic Loci for Little Lost Man Creek and . . Lost Man Creek	51
G Estimates of Gametic Disequilibrium Among Diagnostic Loci for Prairie Creek Tributaries . . and Redwood Creek Embayment	52
H Estimates of Gametic Disequilibrium among Diagnostic Loci for each Species Group in Redwood Creek Tributaries	53

TABLE OF CONTENTS (continued)

vii

I Estimates of Gametic Disequilibrium among
 Diagnostic Loci for each Species Group in
 Little Lost Man Creek and Lost Man Creek 54

J Estimates of Gametic Disequilibrium among
 Diagnostic Loci for each Species Group in
 Prairie Creek Tributaries 55

K Estimates of Gametic Disequilibrium among
 Diagnostic Loci for each Species Group and
 Age-Class Group in Redwood Creek Embayment 56

LIST OF TABLES

Table		Page
1	Redwood Creek Tributaries Sampled	4
2	Buffer Formulas Used to Resolve Enzymes	11
3	Enzymes Investigated in Redwood Creek Trout	12
4	Allele Frequencies at Species Discriminating Loci . Used in Hybrid Index	19
5	Goodness of fit to Hardy-Weinberg genotypic proportions for each sample site in Redwood Creek, California.	25
6	Goodness of fit to gametic equilibrium in tributaries in Redwood Creek, California that . . appear to be single populations of trout.	27
7	Goodness of fit to Hardy-Weinberg genotypic proportions for each sub-population of trout in . tributaries to Redwood Creek, California.	28
8	Goodness of fit to gametic equilibrium in sub-populations of trout in tributaries to Redwood Creek, California.	30
9	Trout Population Descriptions for Redwood Creek Tributaries, California	33

LIST OF FIGURES

Figure		Page
1	Map of Lower Redwood Creek, California	3
2	Distribution of Hybrid Index Scores for Redwood Creek Tributaries	20
3	Distribution of Hybrid Index Scores for Little Lost Man Creek and Lost Man Creek	21
4	Distribution of Hybrid Index Scores for Prairie Creek Tributaries	22
5	Distribution of Hybrid Index Scores for Redwood Creek Embayment	23

INTRODUCTION

Coastal cutthroat (Oncorhynchus clarki) inhabit the west coast of North America from the Eel River in Northern California (Dewitt 1954) to the Prince William Sound area near Seward, Alaska (Scott and Crossman 1973). Hell's Gate on the Fraser River and Celilo Falls on the Columbia River (now inundated by Dalles Dam reservoir) are generally accepted as the inland distributional limits of the coastal cutthroat species in Western North America (Carl et al. 1959; Crawford 1979). Anadromy tends to be poorly developed (Hoar 1976) and anadromous populations will coexist with resident populations throughout its geographical range. In the Pacific Northwest anadromous cutthroat are generally referred to as "sea-runs" hence the name, sea-run coastal cutthroat trout.

Management agencies in the Pacific Northwest have historically overlooked the recreational importance of "sea-runs" because of the economic value and publicity given to steelhead trout (Oncorhynchus mykiss) and the five indigenous species of Pacific salmon (Oncorhynchus 5 sp.). In contrast to steelhead trout and Pacific salmon, cutthroat trout rarely venture into the open ocean on their migrations but instead remain close to shore in association with river mouths and other estuarine areas (Haig-Brown 1947; Royal 1972; Johnston and Mercer 1976). As a result, cutthroat trout provide unique angling opportunities that are not available with the other salmonid fishes in the Pacific Northwest.

One of the streams in northern California with a population of coastal cutthroat trout is Redwood Creek. Redwood Creek basin, located about 64 kilometers (40 miles) northeast of Eureka, California (Figure 1), is about 725 square kilometers (280 square miles) of rugged and highly erosive terrain within the North Coast Ranges of California. The basin (with the exception of Prairie Creek) is strongly elongated in a north-northwesterly direction and is about 90 kilometers (56 miles) long, and 7.2 to 11.1 kilometers (4.5 to 6.9 miles) wide throughout most of the basin. The altitude ranges from sea level at the mouth to 1,615 meters (5,298 feet) at the headwaters of the basin (Iwatsubo and Averett 1981).

Prairie Creek, the largest and downstream-most of Redwood Creek's major tributaries, drains about 104 square kilometers (40 square miles) and enters the main creek approximately 6.1 (3.8 miles) river miles from the sea. Its altitude ranges from sea level to about 305 kilometers (1,000 feet) and has a length approximately 22.5 kilometers (14 miles).

The information available on coastal cutthroat trout in Redwood Creek, California is rather limited but implies that life history patterns for this species are variable (unpublished data, Redwood National Park, Arcata, CA 95521). Smolts in anadromous populations typically descend to an estuarine or coastal embayment during spring to early summer at age II or III and at a size of 11-23 centimeters

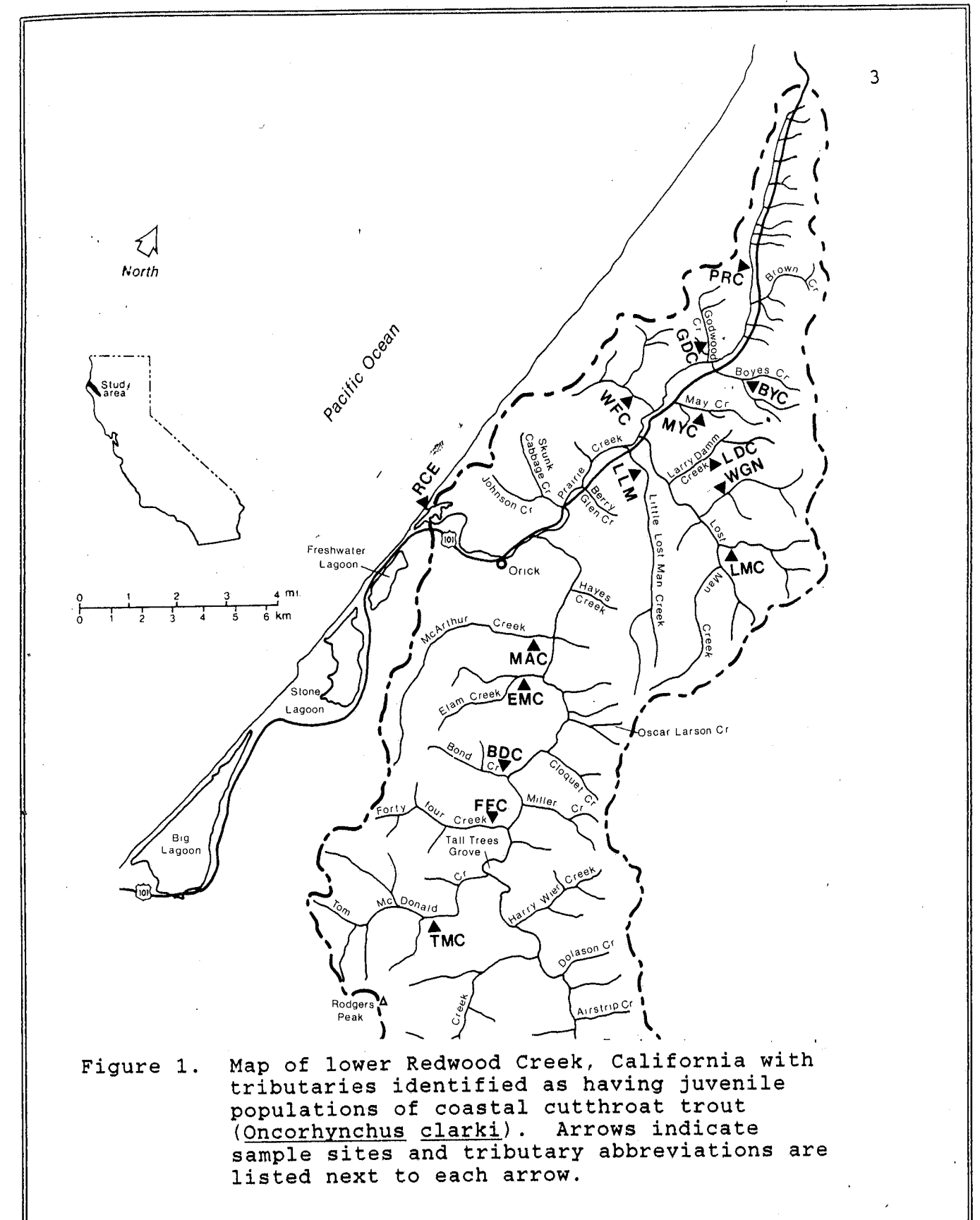


Table 1. Redwood Creek tributaries sampled for coastal cutthroat trout and steelhead trout. Stream abbreviation is in parenthesis.

#	Drainage/Stream Sample Site	
	Redwood Creek Basin	
1	Tom McDonald Creek	(TMC)
2	Forty-Four Creek	(FFC)
3	Bond Creek	(BDC)
4	Elam Creek	(EMC)
5	MacArthur Creek	(MAC)
	Prairie Creek Basin	
6	Little Lost Man Creek	(LLM)
	Lost Man Creek Basin	
7	Larry Dam Creek	(LDC)
8	Wigeon Creek	(WGN)
9	Lost Man Creek	(LMC)
10	May Creek	(MYC)
11	Boyes Creek	(BYC)
12	Headwaters Prairie Creek	(PRC)
13	Godwood Creek	(GDC)
14	Wolf Creek	(WFC)
15	Redwood Creek Embayment	(RCE)

(unpublished data, Redwood National Park). Outmigrants evidently do not travel in the open ocean but rather concentrate in Redwood Creek embayment and along the coast and return the same year in late summer or fall. Maximum age of sea-run coastal cutthroat trout observed in Redwood Creek was 6+, with three-to five-year-old trout most abundant.

Several types of life history patterns associated with non-anadromous coastal cutthroat trout were exhibited by fish in Redwood Creek drainage (unpublished data, Redwood National Park):

- (1) Fish that are born in small tributaries, leave after one or two years, to rear in larger streams, migrating each winter to the small tributaries for spawning.
- (2) Fish that are born in large streams and spend their entire life cycle in these same streams.
- (3) Fish that are born in small tributaries and spend their entire life cycle in the same tributary (not restricted to populations above barriers).

Few resident coastal cutthroat trout live more than five years in Redwood Creek, with two-to three-year-olds most abundant (unpublished data, Redwood National Park).

All anadromous populations within Redwood Creek have declined from historical levels (unpublished data, Redwood

National Park, Arcata, CA 95521). This has been due in large part to poor land management activities in the upper watershed and the "flood control project" (channelization and levees) by the U.S. Army Corps of Engineers on the lower 6.4 kilometers of Redwood Creek. Whenever management and enhancement of sea-run coastal cutthroat trout is discussed, the question arises as to "which tributaries contribute sea-run coastal cutthroat trout to the anadromous population in Redwood Creek embayment?" The only information available are tributaries that have been identified as having juvenile cutthroat trout residing in them (see Brown 1988).

The sea-run coastal cutthroat trout in Redwood Creek are potentially recruited from any tributary with spawning cutthroats. To insure that numerically small stocks are not overharvested and/or numerically large stocks are not underharvested that are contributing to the mixed stock fishery (sea-run cutthroat trout) in Redwood Creek, it is essential that knowledge of stock contribution be assessed to effectively manage the fishery (Larkin 1981). Stock composition strategies have been rarely practiced due to difficulty in adequately identifying stock groups that contribute to a fishery.

The general absence or difficulty in identifying natural marks have led to artificial marking procedures, such as coded wire tagging and fin clipping, which have become valuable fishery management tools for identifying specific stocks of hatchery fish. Use of artificial marking

procedures on wild populations requires considerable more effort and cost (Ihssen et al. 1981).

More complicated procedures have been used to detect natural marks in fish stocks, such as scale analysis (Messinger and Bilton 1974), parasite infestations (Margolis 1963), morphometric and meristic characters (Amos et al. 1963; Fukuhura et al. 1962), elemental chemical composition (Mulligan et al. 1983), and genetic characters (Ridgeway et al. 1962). Genetic characters are the only procedure unaffected by environmental conditions, and do not require yearly examination and revision of standards.

The biochemical genetic stock identification of several Oncorhynchus species and the effective management of Oncorhynchus tshawytscha in the Pacific Northwest (Grant et al. 1980; Fournier et al. 1984; Beacham et al. 1985 a,b; Resienbichler and Phelps 1985; Milner et al. 1985; Utter et al. 1987) suggests the genetic stock identification (GSI) method could be utilized to determine contributing tributary stocks to the sea-run coastal cutthroat trout fishery in Redwood Creek.

Two conditions must be met to utilize the genetic stock identification method. First, each tributary stock that could contribute the sea-run cutthroat trout fishery must be electrophoretically characterized. Second, to measure contributions from each stock, sufficient differences among the genetic profiles must exist and be identified. Pella and Milner (1987) provide a formal discussion of these

general principles and the mathematical bases for analyzing the contributing components of stock mixtures.

The natural hybridization between steelhead trout, Oncorhynchus mykiss, and coastal cutthroat trout, Oncorhynchus clarki, would interfere with characterizing tributary stocks of coastal cutthroat trout in Redwood Creek. The apparent natural hybridization between these congeners in two Puget Sound streams (Campton and Utter 1985) suggests that natural hybridization is possible in Redwood Creek.

In recent years protein electrophoresis has been a reliable technique for identifying hybrid fish (e.g. Nyman 1970; Reinitz 1977; Danzmann and Down 1982; Leary et al. 1984; Campton and Utter 1985). The technique has proven valuable for detecting natural hybridization between inland forms of cutthroat trout and non-native rainbow trout (Allendorf and Phelps 1981; Busack and Gall 1981) and intraspecific hybridization between native and introduced populations of rainbow trout (Allendorf et al. 1980; Wishard et al. 1984; Campton and Johnston 1985).

In this paper, I discuss my use of protein electrophoresis to examine the potential natural hybridization between Oncorhynchus mykiss and Oncorhynchus clarki residing in Redwood Creek watershed. I then consider the biological and management implications of my findings.

METHODS

Cutthroat trout were collected for genetic analysis from 15 sample sites within Redwood Creek (Table 1). Nine of these streams are located in the Prairie Creek drainage (Figure 1), while five streams are located upstream of Prairie Creek on Redwood Creek's western half of its watershed. Redwood Creek's embayment was also sampled.

In general, two separate age-class groups of fish (age 2+ and 3+) were collected from all sample sites. An effort was made to collect 30 fish from each stream (July-August, 1984) and 50 fish from Redwood Creek embayment over a two year period, 1984 through 1985.

All fish were collected in the streams with a backpack electroshocker. All fish in Redwood Creek embayment were collected with Redwood National Park's 50 meter Beach seine. Trout were identified, fork length measured, and killed by being placed in plastic bags and immediately placed on a wet ice bath in an ice chest. They were then transported to the laboratory where they were frozen at approximately -25 degrees centigrade for no longer than six months before being analyzed electrophoretically.

Electrophoresis

Horizontal starch gel electrophoresis was performed on liver, eye, heart, and muscle tissues as outlined by Utter

et al. (1974) and May et al. (1979). Three buffer systems were used to resolve trout isozymes on starch gels (Table 2). Fourteen enzyme systems representing 23 biochemical loci were examined in all populations sampled (Table 3). Following electrophoresis, each gel was horizontally sliced into three slabs and each slab was placed in 150 milliliters of the appropriate stain solution. Genotypes for each fish were recorded according to the nomenclature systems proposed by Kendall (1989). Staining methods followed standard procedures and are outlined in a manual (Aebersold et al. 1987).

Several isozyme systems in salmonid fishes are represented by duplicated loci (IDHP-3,4; MDH-3,4; MEP-1,2) with common allelic forms of identical electrophoretic mobilities (Allendorf et al. 1975; May et al. 1979). They were treated as two disomic loci with identical allele frequencies because allelic variation could not be attributed to specific forms.

Data Analysis

Two analytical approaches are used to determine if steelhead and cutthroat trout are hybridizing in the Redwood Creek drainage. One method is the hybrid index (Campton and Utter 1985) which estimates the probability of pure species parentage. The second method evaluates the breeding

Table 2. Buffer systems used for electrophoresis of trout samples from Redwood Creek, California.

Buffer	Stock solution	Dilution Factor
Ridgeway et al. (1970)	<u>Gel pH 8.5</u>	
	0.03 M Tris (hydroxymethyl)- aminomethane	7.27g/2L
	0.004 M Citric acid	1.5 g/2L
	<u>Electrode pH 8.2</u>	
	0.06 M LiOH-H ₂ O	5.04g/2L
	0.30 M Boric acid	37.10g/2L

gel buffer consists of 99% gel buffer and 1% electrode

Tris-phosphate

<u>Gel-electrode pH 8.2</u>	
0.10 M Tris (hydroxymethyl)- aminomethane	24.22g/2L
0.037 M NaH ₂ PO ₄ -H ₂ O	18.63g/2L

use concentrated for electrode buffer and dilute 50 milliliters of concentrated to 500 milliliters with distilled water for gel buffer

Amine citrate

<u>Gel-electrode pH 6.5</u>	
0.04 M Citric acid	15.38g/2L
N(3-aminopropyl) morpholine	27ml/2L

use concentrated for electrode buffer and dilute 50 milliliters of concentrated buffer to 500 milliliters with distilled water for gel buffer

Table 3. Proteins examined and corresponding buffer used in trout samples from Redwood Creek, California.

Enzyme	Abbreviation	Enzyme Number	Number of loci	Buffer	Tissue
Alcohol dehydrogenase	ADH	3.5.4.4	1	1 ^a	Liver
Creatine kinase	CK	2.7.3.2	2	1	Muscle
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	2	3 ^b	Muscle
Glucose-6-phosphate isomerase	GPI	5.3.1.9	3	1	Muscle
L-iditol dehydrogenase	IDDH	1.1.1.14	2	1	Liver
Isocitrate dehydrogenase	IDHP	1.1.1.42	2	3	Liver
L-lactate dehydrogenase	LDH	1.1.1.27	1	1	Liver
Malate dehydrogenase	MDH	1.1.1.37	4	3	Heart
Malic enzyme	MEP	1.1.1.40	2	3	Liver
Mannose-6-phosphate isomerase	MPI	5.3.1.8	1	1	Eye
Dipeptidase	PEPA	3.4.13.11	1	2 ^c	Muscle
Tripeptide amino-peptidase	PEPB	3.4.11.4	1	2	Muscle
Phosphoglucosmutase	PGM	5.4.2.2	1	3	Liver
Phosphogluconate dehydrogenase	PGDH	1.1.1.44	1	3	Liver
Superoxide dismutase	SOD	1.15.1.1	1	1	Liver

a Ridgeway et al. (1970) buffer

b Amine-citrate buffer

c Tris-phosphate buffer

structure of the populations sampled statistically as a deviation from a theoretical, single random mating population.

The reliability of detecting hybridization using electrophoretic methods is a function of the extent of genetic differentiation of the parental species. If species are fixed for different alleles at one or more loci, then each individual fish can be unambiguously classified as to their parentage. Parental species will express homozygous phenotypes for different alleles, whereas hybrids will be heterozygous at all diagnostic loci. If hybridization has proceeded past the first-generation (F_1), then hybrid descendants will express a broad mixture of recombinant types, including the parental types. The expression of a broad mixture of recombinant types can be interpreted as evidence of hybrid fertility and second-generation hybrids. If the parental species are fixed for different alleles at only one locus, then first-generation hybrids cannot be distinguished from backcross or second-generation hybrids.

If the two species are not fixed for different common alleles at one or more loci, then each individual fish cannot be unambiguously classified as to their parentage. A hybrid index devised by Campton and Utter (1985) was used to quantitatively demonstrate the likelihood of hybridization. The index measures the relative probability that the composite genotype for each fish arose by random mating

within each of the two species. The index (I_H) can assume any value between 0.0 and 1.0 and will be very close to one of these two values when individuals have a relatively high probability of belonging to cutthroat trout or steelhead trout, respectively.

One disadvantage with using the hybrid index method is it requires a priori allele frequency profiles for each parental species. Campton and Utter (1985) used average allele frequencies from hatchery populations of the two species. No pedigreed populations of coastal cutthroat trout were available in Redwood Creek drainage. Consequently, tributary populations that appeared morphologically to be "pure" cutthroat trout, and their average allele frequencies at the distinguishing loci matched up well with allele frequencies used by Campton and Utter (1985), were used in the hybrid index function.

Allele frequency data can be analyzed and interpreted in terms of simple Mendelian genetics in which alleles are codominantly expressed at single loci. The first step is to determine whether more than one population has been sampled. A single, randomly mating population is expected to conform to Hardy-Weinberg proportions at all loci. A mixture of two, non-interbreeding populations with different allele frequencies is expected to yield a deficiency of heterozygotes when compared to Hardy-Weinberg proportions.

A deficiency of heterozygotes will also occur if two populations interbreed to some extent but mate assortatively within populations. The X^2 -statistic is used to test for significance of single locus goodness of fit to Hardy-Weinberg proportions (Sokal and Rohlf 1969). A pooled significance test for multiple statistical tests done simultaneously was used to test for overall goodness of fit to Hardy-Weinberg proportions for fish collected from each sample site (Cooper 1968).

The second step in analyzing allele frequency data is to measure gametic phase disequilibrium, which indicates the residual consequences of hybridization. Gametic phase disequilibrium is defined as the non-random association of alleles between loci (Campton 1987). Gametic disequilibrium can be generated by the sub-division of a population into several subpopulations or the agglomeration of two or more previously isolated populations. In contrast to Hardy-Weinberg proportions which will be restored after one generation of random mating (in the absence of selection), gametic equilibrium will not be restored after a single generation of random mating. Gametic equilibrium is approached asymptotically in a random mating population and at a rate proportional to the recombination rate between loci (Campton 1987).

Gametic disequilibrium among two loci is estimated using Burrow's composite measure (Campton 1987), which does not

require the assumption of a random mating population in Hardy-Weinberg proportions. The composite measure D includes the normal measure of disequilibrium plus an added component due to the non-random union of gametes ($D=D+d$). The hypothesis $H_0: D(D+d)=0$ was tested using the statistic Nr^2 , where r is the estimated correlation between alleles, properly adjusted for departures from Hardy-Weinberg proportions, and N is the sample size (see Campton 1987 for details). The statistic Nr^2 is a chi-square distributed statistic with one degree of freedom (Campton 1987). A pooled significance test for multiple statistical tests made simultaneously was used to test goodness of fit to gametic equilibria for each population sampled (Cooper 1968).

RESULTS

Nineteen of the 23 loci routinely examined in coastal cutthroat trout were polymorphic for two or more alleles in at least one sample (see Appendix A-D). Only four loci (CK-1, G3PDH-1, IDDH-2, PGDH-1) were identically monomorphic in all trout samples. Variation expressed by several polymorphic systems (e.g. ADH, G3PDH-2, GPI-3, MEP-2, LDH-5) are considered insufficient to provide meaningful statistical results. Ten isozyme systems (14 loci) expressed composite variant allele frequencies greater than 0.01: CK-2, GPI-1,2, IDDH-2, IDHP-3,4, MDH-3,4, MEP-3,4, PEPA, PEPB, PGM, and SOD.

Campton and Utter (1985) found that rainbow (steelhead) trout and coastal cutthroat trout expressed different common alleles at the following loci: CK-2, PEPA-1, IDDH-1, IDHP-3,4 and MEP-4. Campton did not use CK-2 to characterize putative hybrids because of difficulties in distinguishing between the hybrid heterozygote, which has an asymmetrical 3 banded phenotype, and the steelhead homozygote which has a symmetrical 3 banded phenotype. The other four distinguishing isozyme systems were clearly expressed and were used to distinguish Oncorhynchus mykiss and Oncorhynchus clarki in the wild population samples from Redwood Creek.

Little Lost Man Creek and Lost Man Creek trout were used to determine average steelhead allele frequencies for input parameters into the hybrid index function (see Table 4). Since there was no evidence of cutthroat trout common alleles, May Creek, Forty-Four Creek, and Bond Creek trout were used to determine average coastal cutthroat trout allele frequencies for input parameters into the hybrid index function. As will be evident later, four steelhead trout were captured in Bond Creek; they were not included in determining coastal cutthroat trout input parameters.

Hybrid index scores for individuals from each tributary are summarized in Figures 2 through 5. Using the criteria suggested by Campton and Utter (1985); individuals with scores less than 0.30 or greater than 0.70 are attributed to mating within individuals of the same parental species, while scores between 0.30 and 0.70 are attributed to matings between individuals of different parental species, or hybrid parents.

Based on these criteria, all individuals in samples from Little Lost Man Creek and Lost Man Creek exhibited very high probabilities of being steelhead trout (Figure 3). Samples from Forty-Four Creek exhibited very high probabilities of individuals being coastal cutthroat trout (Figure 2). Samples from Bond Creek, Elam Creek, Larry Dam Creek, and Redwood Creek embayment exhibited bimodal hybrid scores, indicating populations of non-interbreeding steelhead and coastal cutthroat trout.

Table 4. Allele frequencies at species discriminating loci for wild populations of Oncorhynchus mykiss and Oncorhynchus clarki in Redwood Creek, CA. Average allele frequencies for the two species were calculated as the unweighted (by sample size) arithmetic averages of the allele frequencies for the respective populations.

Loci	Allele	SH-LLM	SH-LMC	AVG	CT-MYC	CT-FFC	CT-BDC	AVG
IDDH-2	135				.938	.719	.900	.852
	100	1.000	1.000	1.000	.062	.281	.100	.148
IDHP-3,4	170	.056		.028				
	140	.618	.642	.630	.432	.507	.400	.446
	115	.007		.004				
	100	.160	.342	.251	.545	.414	.600	.520
	90	.007		.004		.006		.002
	60	.153	.016	.084	.023	.072		.032
MEP-4	125				.912	.526	1.000	.813
	110					.474		.158
	100	1.000	1.000	1.000	.088			.029
PEPA	110				.912	1.000	1.000	.971
	100	1.000	1.000	1.000	.088			.029

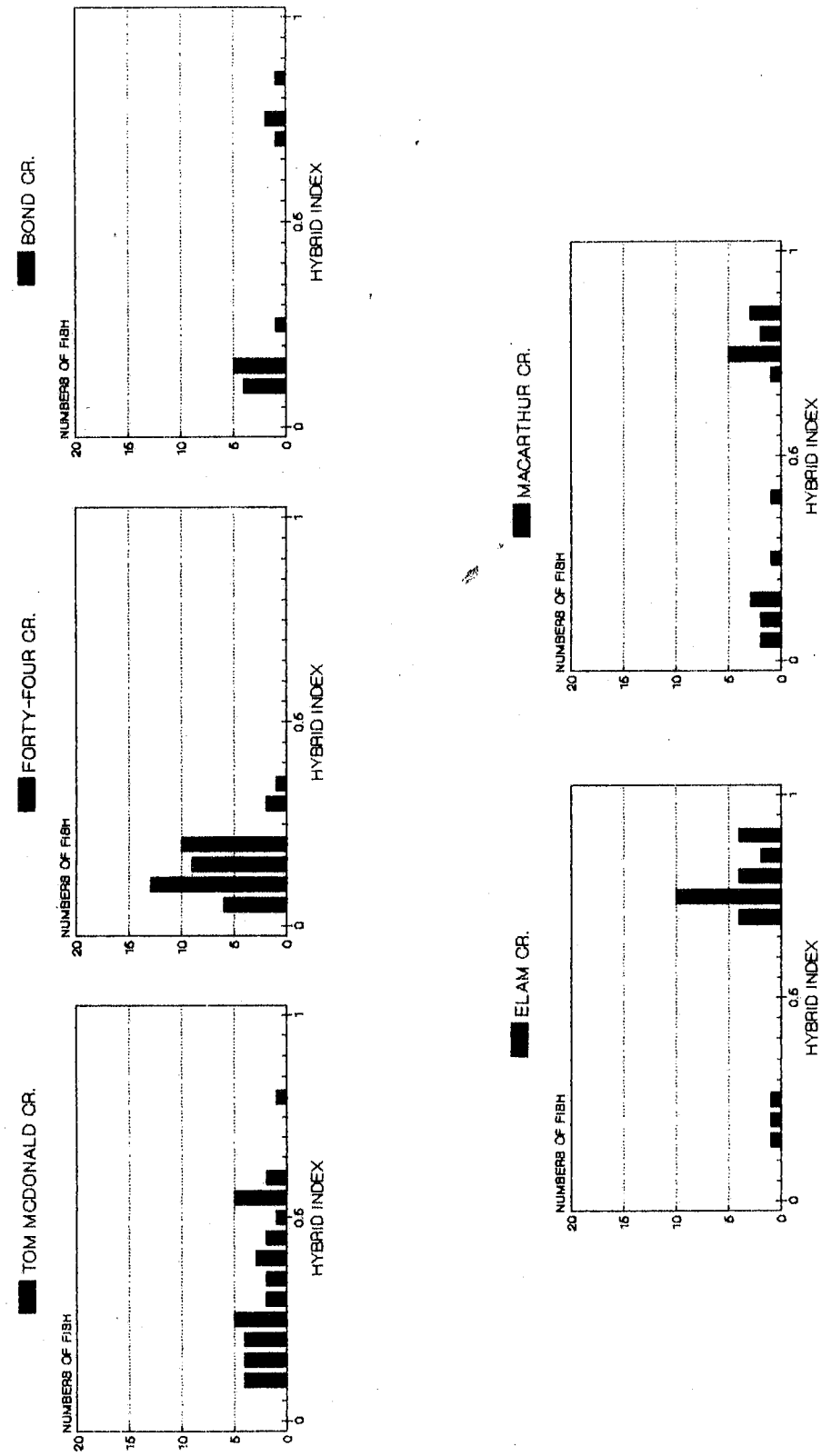


Figure 2. Distribution of hybrid index scores (I_H) for the tributaries in Redwood Creek, CA. Index scores close to 0.0 or 1.0 indicate a very high probability that the individual is either a cutthroat trout or a steelhead trout, respectively.

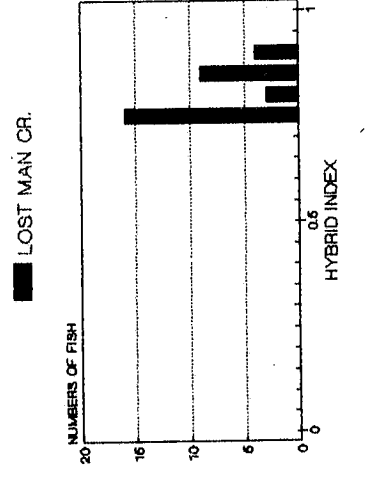
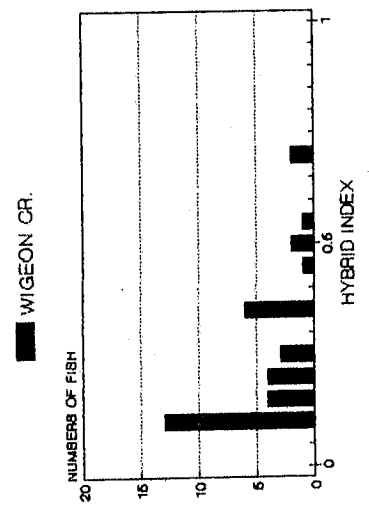
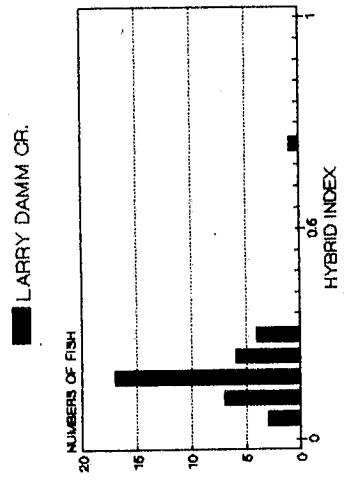
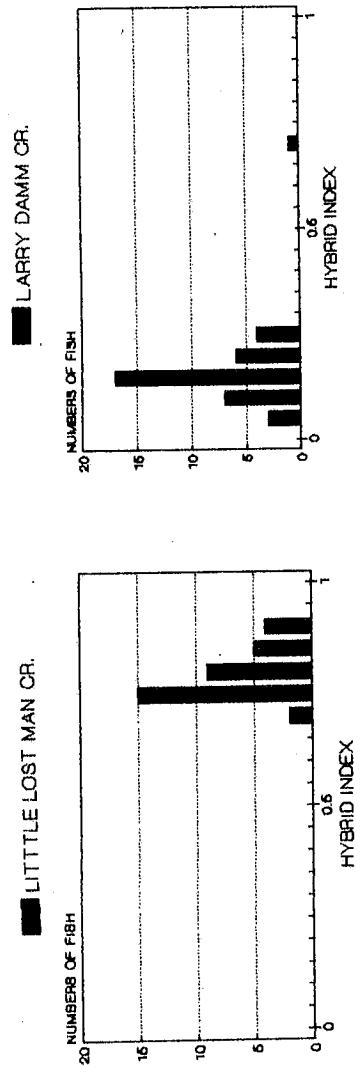


Figure 3. Distribution of hybrid index scores (I_H) for Little Lost Man Creek and Lost Man Creek. Index scores close to 0.0 or 1.0 indicate a very high probability that the individual is either a cutthroat trout or a steelhead trout, respectively.

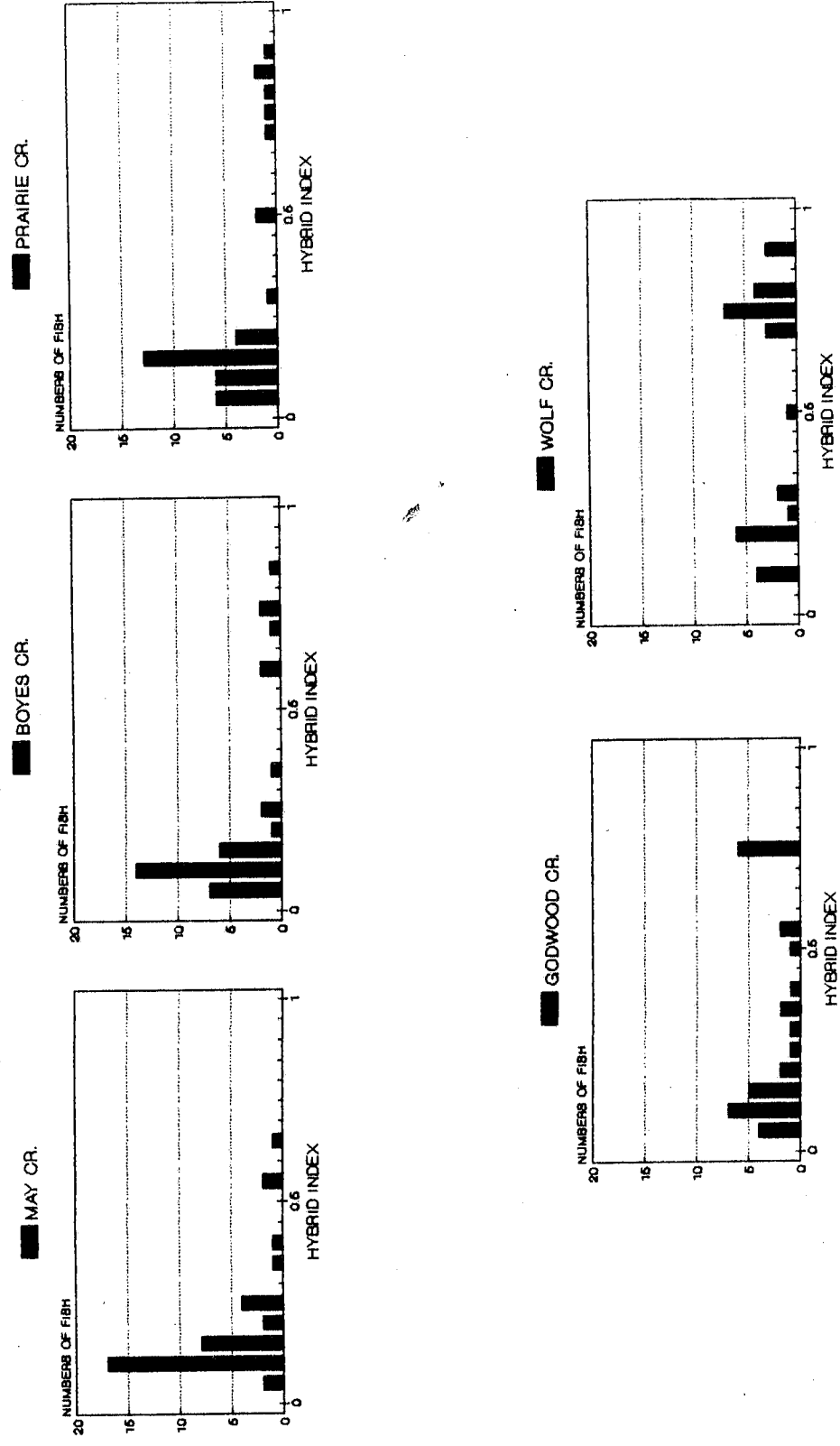


Figure 4. Distribution of hybrid index scores (I_h) for Prairie Creek tributaries. Index scores close to 0.0 or 1.0 indicate a very high probability that the individual is either a cutthroat trout or a steelhead trout, respectively.

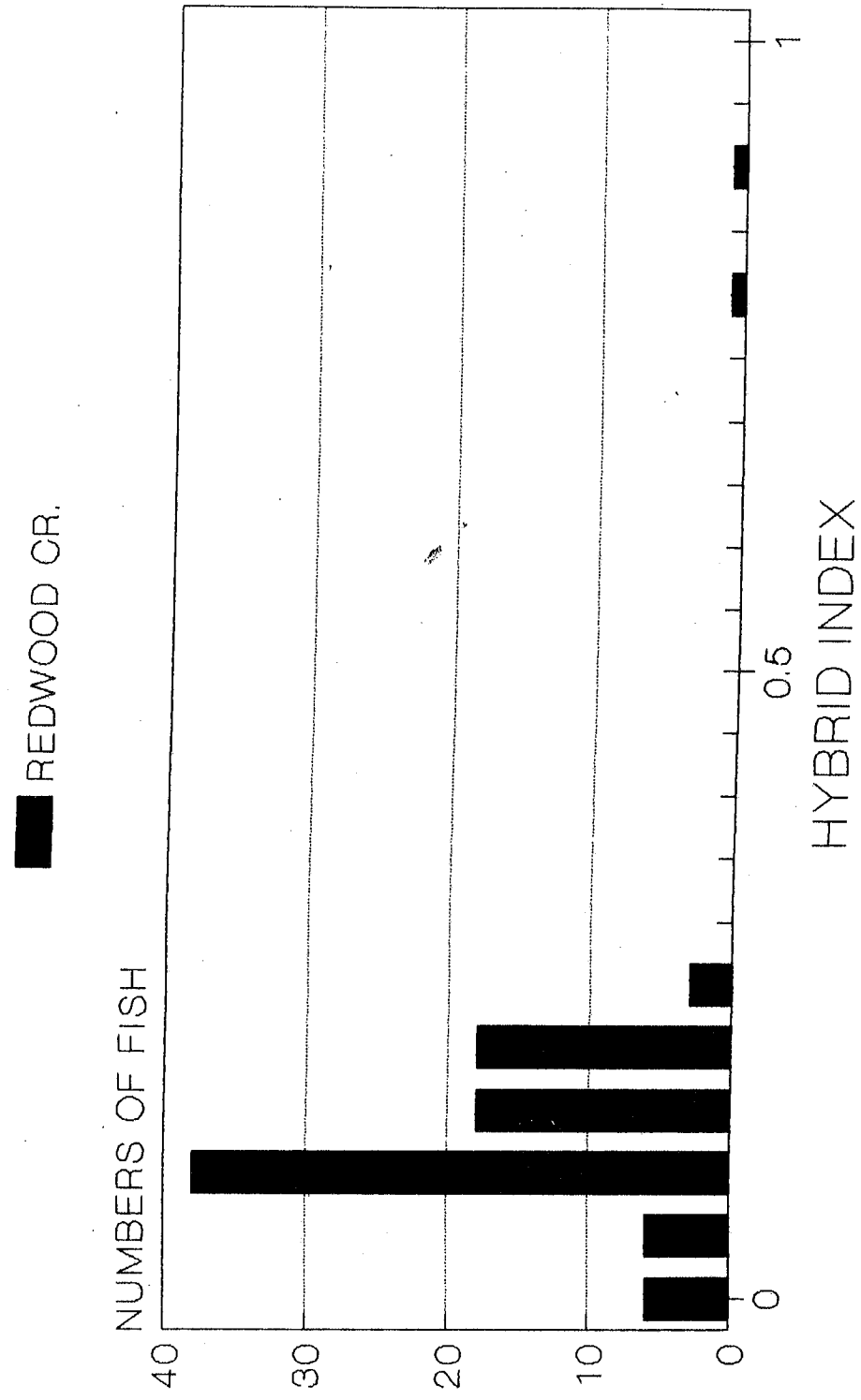


Figure 5. Distribution of hybrid index scores (I_h) for Redwood Creek embayment. Index scores close to 0.0 or 1.0 indicate a very high probability that the individual is either a cutthroat trout or a steelhead trout, respectively.

Individuals from May Creek sample had high probabilities of being coastal cutthroat trout while 4 fish had probabilities of being hybrids. No samples contained probabilities of being generally steelhead trout in conjunction with probabilities of a few hybrids.

Samples from MacArthur Creek, Boyes Creek, Prairie Creek and Wolf Creek exhibited bimodal probabilities reflecting generally distinct populations of steelhead trout and coastal cutthroat trout, but with a few hybrids present.

Samples from Tom McDonald Creek, Wigeon Creek, and Godwood Creek exhibited hybrid index scores that reflected three distinct populations of trout; steelhead trout, coastal cutthroat trout, and hybrids.

Goodness of fit to Hardy-Weinberg genotypic proportions are presented in Table 5. A sample is considered to be out of Hardy-Weinberg equilibrium if the pooled value from all distinguishing loci are significant. On this basis, samples from Little Lost Man and Lost Man Creek appear to be each a single population of steelhead trout. Tom McDonald Creek sample appears to be a single population of hybridized trout. Forty-Four Creek sample exhibited a highly significant deficit of heterozygotes between the two cutthroat alleles at the MEP-4 locus, but appears otherwise to be a single population of coastal cutthroat trout. All other tributaries sampled appear to be comprised of more than one distinct population of trout.

Table 5. Goodness of fit to Hardy-Weinberg genotypic proportions at the three distinguishing loci for each sample site in Redwood Creek, California, and the pooled estimate of significance using the χ^2 -statistic. ** $P < .01$; *** $P < .001$.

Location	Sample Size	IDDH-2	Loci MEP-4	PEPA	Pooled
TMC	34	7.1**	0.2	3.2	10.5
FFC	36	0.1	15.7***	0.0	15.8***
BDC	14	10.0**	0.3	14.0***	24.3***
EMC	30		31.0***	20.1***	51.1***
MAC	19	6.7**	25.4***	19.0***	51.0***
LLM	36	0.0	0.0	0.0	0.0
LDC	38	20.0***	22.5***	8.1**	50.6***
WGN	35	3.0	13.3**	8.9**	25.2***
LMC	30	0.0	0.0	0.0	0.0
MYC	36	1.8	9.3**	3.4	14.5**
BYC	38	1.0	13.1***	9.0**	23.1***
PRC	38	21.8***	20.7***	9.6**	52.1***
GDC	33	6.4	26.9***	24.6***	57.9***
WFC	32	17.8***	0.3	30.0***	48.1***
RCE	64	15.4***	43.4***	80.0***	138.8***

Estimates of gametic (linkage) disequilibrium (Table 6) among IDDH-2, MEP-4, and PEPA for each sample that was in Hardy-Weinberg equilibrium was used to detect residual evidence of introgression between Oncorhynchus mykiss and Oncorhynchus clarki in Redwood Creek tributaries.

Estimates of disequilibrium were not significantly different than zero in Forty-Four Creek, Little Lost Man Creek, and Lost Man Creek. Tom McDonald Creek was significantly different than zero in two out of three loci combinations and in the pooled estimate.

Each sample exhibiting significant departure from Hardy-Weinberg equilibrium was then partitioned into three sub-populations based on hybrid index scores to further evaluate hybridization and introgression. Fish with scores less than 0.30, greater than 0.70, and between the two values have been provisionally classified as "cutthroat" trout (CT), "steelhead" trout (SH), or as "hybrids" (HB), respectively (see Figures 2-5).

Based on these criteria, all sub-populations of coastal cutthroat trout appeared to be in Hardy-Weinberg equilibrium, with the exception of Larry Dam and Wigeon Creek (Table 7). Larry Dam Creek cutthroat trout exhibited a highly significant deficit of heterozygotes between the two cutthroat alleles at the MEP-4 locus ($P < .001$). Wigeon Creek cutthroat trout exhibited a highly significant excess of heterozygotes at the MEP-4 locus ($P < .001$). Sub-populations

Table 6. Goodness of fit to gametic equilibrium in Redwood Creek, California tributaries that appeared to be a single population of trout. The X^2 -statistic was used to test the hypothesis that $H_0: D=D+d=0$, where $X^2=Nr^2$, N is the sample size, and r is the estimated correlation between alleles. Loci numbers 1, 2, and 3 refer to loci PEPA, IDDH-2, and MEP-4, respectively. ** $P<.01$; *** $P<.001$.

Location	Sample Size	Loci Comparisons ^a			Pooled
		(1,2)	(1,3)	(2,3)	
TMC	34	0.4	6.6	13.6***	20.6***
FFC	36	0.1	0.0	0.0	0.1
LLM	36	0.0	0.0	0.0	0.0
LMC	30	0.0	0.0	0.0	0.0

a Estimates of gametic equilibrium (D) and the correlation between alleles (r) are tabulated in Appendixes E-G.

Table 7. Goodness of fit to Hardy-Weinberg proportions for each sub-population of trout in tributaries with mixed species from Redwood Creek, California. The χ^2 -statistic was used to test individual loci and the pooled estimate. ** $P < .01$; *** $P < .001$.

Location	Species	Sample Size ^a	Loci			
			IDDH-2	MEP-4	PEPA	Pooled
BDC	CT	9	0.0	2.0	0.0	2.0
	SH	1	0.0	0.0	0.0	0.0
EMC	CT	3	0.0	0.0	0.0	0.0
	SH	28	0.0	0.0	0.0	0.0
MAC	CT	8	0.0	2.0	0.0	2.0
	HB	1	0.0	0.0	0.0	0.0
	SH	12	0.0	0.0	0.0	0.0
LDC	CT	35	2.0	26.0	0.0	28.0***
	SH	1	0.0	0.0	0.0	0.0
WGN	CT	23	2.0	12.0	0.0	14.0**
	HB	11	1.0	5.0	0.0	6.0
	SH	2	0.0	0.0	0.0	0.0
MYC	CT	35	0.0	0.0	1.0	1.0
	HB	5	1.0	1.0	0.0	2.0
BYC	CT	30	0.0	0.0	1.0	1.0
	HB	3	0.0	11.0	0.0	11.0
	SH	4	0.0	0.0	0.0	0.0
PRC	CT	28	3.0	0.0	1.0	4.0
	HB	3	0.0	0.0	0.0	0.0
	SH	5	0.0	0.0	0.0	0.0
GDC	CT	18	0.0	0.0	0.0	0.0
	HB	8	10.0	18.0	6.0	34.0***
	SH	6	0.0	0.0	0.0	0.0
WFC	CT	11	0.0	0.0	0.0	0.0
	HB	3	0.0	0.0	0.0	0.0
	SH	18	0.0	0.0	0.0	0.0
RCE	CT 2+	7	0.0	0.0	0.0	0.0
	3+	68	6.0	2.0	0.0	8.0
	4+	13	0.0	0.0	0.0	0.0
	SH 3+	2	0.0	0.0	0.0	0.0

^a Generally it is very hard to identify significant deviations from Hardy-Weinberg equilibrium when sample sizes are small.

of hybrid trout in Godwood Creek also appear to differ significantly from Hardy-Weinberg equilibrium ($P < .001$). All sub-populations of steelhead trout appeared to be in Hardy-Weinberg equilibrium.

Gametic (linkage) disequilibrium was estimated among the diagnostic loci for each sub-population of trout and tested for significance against the null hypothesis ($H_0: D = D + d = 0$), no gametic disequilibrium (Table 8). Pooled estimates of disequilibrium were significantly different from zero for cutthroat trout sub-populations in Larry Dam Creek, Wigeon Creek, May Creek, Godwood Creek, and Wolf Creek. Wigeon Creek's pooled estimate of significance for the hybrid trout sub-population was highly significant despite the small sample size. Pooled estimates of significance for gametic disequilibrium were not significantly different from zero in all steelhead sub-populations.

In summary, single populations of steelhead trout were identified in Little Lost Man Creek and in Lost Man Creek. A single population of coastal cutthroat trout was identified in Forty-Four Creek and a single population of hybrid trout was identified in Tom McDonald Creek. May Creek appeared to be a single population of cutthroat trout that has been recently introgressed by steelhead trout. The remaining tributaries sampled were comprised of a mixture of the two species.

Table 8. Goodness of fit to gametic equilibrium in sub-populations of trout in Redwood Creek, California tributaries. The X^2 statistic was used to test the hypothesis that $H_0: D=D+d=0$, where $X^2=Nr^2$, N is the sample size, and r is the estimated correlation between alleles. Loci numbers 1, 2, and 3 refer to loci PEPA, IDDH-2, and MEP-4, respectively. ** $P<.01$; *** $P<.001$.

Location	Species	Sample Size ^a	Loci Comparisons ^b			Pooled
			(1,2)	(1,3)	(2,3)	
BDC	CT	9	0.1	0.0	0.1	0.2
EMC	SH	23	0.0	0.0	0.0	0.0
MAC	SH	12	0.0	0.0	0.0	0.0
LDC	CT	38	25.4	0.1	7.8	33.2***
WGN	CT	23	119.0	18.1	14.9	152.0***
	HB	11	1.9	16.9	0.1	18.9***
MYC	CT	35	13.6	1.2	0.0	14.8**
BYC	CT	30	0.8	2.0	0.0	2.8
PRC	CT	28	0.1	0.5	1.9	2.5
GDC	CT	18	622.0	0.1	4.9	627.0***
WFC	CT	11	2.9	0.0	13.8	16.7***
	SH	18	0.0	0.0	0.0	0.0
RCE	CT 4+	13	0.2	0.0	0.1	0.3
	3+	68	0.0	0.1	0.0	0.0

a It is very hard to identify significant gametic disequilibria when sample sizes are small.

b Estimates of gametic equilibrium (D) and the correlation between alleles (r) for trout sub-populations are tabulated in Appendixes H-K.

Distinct populations of non-hybridizing steelhead trout and coastal cutthroat trout were identified in Bond Creek, Elam Creek, and in Redwood Creek's embayment. In MacArthur, Larry Dam, Wigeon, Boyes, Prairie, Godwood and Wolf Creeks distinct populations of both species were identified, but with hybridization and/or introgression between Oncorhynchus mykiss and Oncorhynchus clarki.

DISCUSSION and CONCLUSIONS

Prior to sampling individuals from wild populations and subsequent electrophoretic evaluation it is unknown if natural hybridization has occurred. Several situations are possible:

- (1) A single trout population from a single species.
- (2) A single population that has been introgressed by another species (or population).
- (3) A mixture of two or more species (or populations) with no hybridization or introgression.
- (4) A mixture of two or more species (or populations) with hybridization or introgression.

Based on the distribution of the hybrid index scores and the analytical data I am able to classify sample locations into one of these 4 classifications (see Table 9). Only six sample locations out of fifteen exhibited no hybridization or introgression between these congeners. One location, Tom McDonald Creek, appeared to be a randomly mating population of hybrid trout. Two more locations, Wigeon Creek and Godwood Creek, were populated predominantly by individuals with evidence of hybridization and introgression.

These data are used as convincing evidence of natural hybridization between Oncorhynchus mykiss and Oncorhynchus clarki throughout Redwood Creek drainage. However, the presence of fish with common alleles of both parental species can never constitute proof of hybridization. Rather,

Table 9. Trout population descriptions for all sample locations in Redwood Creek, California. Individuals were provisionally classified as either steelhead trout (SH), cutthroat trout (CT), or as "hybrids" (HB) if their hybrid index score was greater than 0.70, less than 0.30, or between the two values, respectively.

Tributary/Location	#CT	#HB	#SH	%HB
<u>Single trout populations</u>				
Little Lost Man Cr.	0	0	36	0%
Lost Man Cr.	0	0	30	0%
Forty-Four Cr.	38	0	0	0%
<u>Single trout population that has been introgressed</u>				
Tom McDonald Cr.	15	19	0	56%
May Cr.	32	4	0	12%
<u>Population mixture with no hybridization or introgression</u>				
Bond Cr.	10	0	4	0%
MacArthur Cr.	8	1	11	5%
Elam Creek	3	0	26	0%
Redwood Creek Embayment	58	0	2	0%
<u>Population mixture with hybridization or introgression</u>				
Larry Dam Creek	38	0	1	0%
Wigeon Creek	24	8	2	24%
Boyes Cr.	28	3	4	9%
Prairie Creek	29	3	6	8%
Godwood Cr.	19	7	6	22%
Wolf Creek	12	3	18	9%

hybridization is the most plausible explanation for the relatively high numbers of fish with intermediate hybrid index scores. Intraspecific genetic variation within Oncorhynchus clarki alone can never be excluded as the source of the intermediate phenotypes, but the probability of producing large numbers of electrophoretically intermediate fish at separate locations by random mating alone is extremely remote.

The extent of backcrossing and introgression resulting from natural hybridization between Oncorhynchus mykiss and Oncorhynchus clarki in Redwood Creek watershed is difficult to ascertain. Backcross or F_2 (second generation) progeny cannot be individually distinguished from F_1 (first generation) hybrids because one or both of the parental species are naturally polymorphic at the four distinguishing isozyme systems. If most fish classified as "hybrids" were heterozygous at the diagnostic loci, then one could conclude that only F_1 hybrids had been sampled. However, an excess of heterozygotes was not consistently observed in trout from Redwood Creek. The preferential expression of maternal or paternal isozymes in trout hybrids (e.g. Hitzeroth et al. 1968; Ohno 1969; Engel et al. 1977) could produce F_1 hybrid phenotypes suggestive of a backcross or F_1 mating. Analysis of progeny from experimental matings between the two species are required to resolve these questions.

The mechanisms preventing widespread loss of species integreties between these two trout are not well understood. The ability of these two closely related congeners to coexist sympatrically and yet maintain their species integreties has generally been attributed to the spatial and temporal separation of spawning adults (Needham and Gard 1959; Behnke 1972, 1979). Cutthroat trout usually spawn in small, low-gradient creeks or in the upper low-flow reaches of large creeks whereas steelhead trout usually spawn in larger, swifter flowing streams (Hartman and Gill 1968). Nevertheless, the time and place of spawning for the two species overlap considerably in the Redwood Creek drainage.

The existence of juvenile "hybrids" in tributary streams, and absence of adult hybrids in the estuary raises intriguing questions. As sea-run adults, these two species are easily distinguished by obvious overall physical appearances and would expect F_1 hybrid adults to be identified by some combination of unique intermediate characteristics. Alternatively, a high incidence of mortality and/or migratory disorientation may occur among hybrids following their first couple of years of life. The presence of hybrid trout older than two years of age (2+ and 3+ years) is noteworthy with regards to possible residualized populations of coastal cutthroat and steelhead trout. Steelhead trout and sea-run coastal cutthroat trout exhibit distinct differences in life histories and patterns of saltwater migration (Scott and Crossman 1973; Wydoski and

Whitney 1979). These patterns could conceivably become disrupted in hybrids; such that hybrids fail to return to freshwater in appreciable numbers following their outmigration to saltwater, or fail to migrate at all and become a resident trout.

The freshwater age of coastal cutthroat trout varies substantially from area to area depending on environmental conditions (Royal 1972). Although some resident coastal cutthroat trout spend up to eight years in freshwater without migration, few of them live more than five years in freshwater (Moring and Lantz 1975). For populations in small tributaries, age 3+ is usually the oldest age class (Aho 1977; Lestelle 1978), and apparently only few live to be more than four years old (Wyatt 1959; Cooper 1970; Moring and Lantz 1975; Moring and Hooten 1978). They first mature at age 2+, with the majority of the remainder maturing at age 3+ and 4+ (Summer 1948; Neave 1949; Donaldson et al. 1957; Wyatt 1959; Hart 1973; Nicholas 1977, 1978a, 1978b; Moring and Hooten 1978).

Hybridization does appear to be widespread geographically but not extensive within streams (Table 9) and species integrities are generally being maintained. This maintenance of species integrities contrasts with the situation for the interior species of cutthroat trout where extensive hybridization follows introductions of non-native rainbow trout.

Anadromy is poorly developed in coastal cutthroat trout (Hoar 1976) and anadromous populations coexist with resident populations throughout its geographical range. Anadromy is more strongly developed in steelhead trout, but resident populations are known to coexist throughout its geographical range (Hoar 1976). Whether coastal cutthroat trout will migrate to sea or remain a freshwater resident form (assuming both have equal access to the ocean) is apparently under genetic control (Meehan 1974). Except for body size, there are no marked differences between sea-run and non-anadromous coastal cutthroat trout (Dimick and Merryfield 1945; Nicholas 1978a). Both forms possess similar life patterns and habits during their freshwater existence.

Natural hybrids between Oncorhynchus mykiss and Oncorhynchus clarki in tributary populations and their absence from the anadromous population in Redwood Creek embayment leads me to hypothesize that hybridization between these two species is more common in their resident populations than it is in the anadromous populations. Natural hybridization between Oncorhynchus mykiss and Oncorhynchus clarki had generally been assumed to be a rare and isolated event (Miller 1950; Needham and Gard 1959; Behnke 1972). Evidence from Redwood Creek and the findings of Campton and Utter (1985) suggest that the production of hybrid offspring may not be uncommon in streams where both species spawn. The existence of sexually mature hybrids in

headwater streams and lack of hybrids in Redwood Creek embayment suggests that species integrities are generally being maintained in the anadromous populations by means of spatial and temporal separation of spawning adults, whereas, in resident populations and/or headwater streams where flows can become intermittent, these barriers may no longer exist and hybridization in these situations may mean survival. Extensive genetic and ecological studies are required to more fully understand the biological interactions between Oncorhynchus mykiss and Oncorhynchus clarki in coastal streams of western North America.

REFERENCES CITED

- Aho, R.S. 1977. A population study of the cutthroat trout in an unshaded and shaded section of stream. M.S. Thesis, Oregon State Univ., Corvallis, OR. 87pp.
- Allendorf, F.W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (Salmo trutta): detection and interpretation from population data. Hereditas 86:179-190.
- Allendorf, F.W., and F.M. Utter. 1979. Population genetics of fish, p. 407-454. In W.S. Hoar, D.S. Randall, and J.R. Brett (ed.) Fish Physiology. Vol. 8. Academic Press, Inc., N.Y.
- Allendorf, F.W., D.M. Espeland, D.T. Scow, and S.R. Phelps. 1980. Coexistence of native and introduced rainbow trout in Kootenai River drainage. Proceedings of the Montana Academy of Sciences 39:28-36.
- Allendorf, F.W., and S.R. Phelps. 1981. Isozymes and the preservation of genetic variation in salmonid fishes, p. 37-52. In N. Ryman (ed.) Fish Gene Pools. Ecol. Bull. No. 34, Stockholm.
- Beacham, T.D., R.E. Withler, and A.P. Gould. 1985a. Biochemical genetic stock identification of chum salmon (Oncorhynchus keta) in southern British Columbia. Can. J. Fish. Aquat. Sci. 42:437-448.
- Beacham, T.D., R.E. Withler, A.P. Gould. 1985b. Biochemical genetic stock identification of pink salmon (Oncorhynchus gorbuscha) in southern British Columbia and Puget Sound. Can. J. Fish. Aquat. Sci. 42:1474-1483.
- Beacham, T.D., A.P. Gould, R.E. Withler, C.B. Murray, and L.W. Barner. 1987. Biochemical genetic stock identification of chum salmon (Oncorhynchus keta) in British Columbia. Can. J. Fish. Aquat. Sci. 44:1702-1713.
- Behnke, R.J. 1972. The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Board Can. 29:639-671.

- . 1979. Monographs of the native trouts of the genus Salmo of western North America. Published jointly by the U.S. Forest Service, U.S. Fish and Wildlife Service, and U.S. Bureau of Land Management. Available from Regional Forester, 11177 W. 8th Ave., P.O. Box 25127, Lakewood, CO 80225, USA, 163pp.
- Brown, R.A. 1988. Physical rearing habitat for anadromous salmonids in the Redwood Creek basin, Humboldt County, California. M.S. Thesis Humboldt State Univ., Arcata, CA. 132 pp.
- Busack, C.A., and G.A.E. Gall. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (Salmo clarki seleniris). Can. J. Fish. Aquat. Sci. 38:552-554.
- Campton, D.E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. Pages 161-192. In N. Ryman and F. Utter (ed.) Population Genetics and Fishery Management. University of Washington Press, Seattle, WA, 420pp.
- Campton, D.E. and J.M. Johnston. 1985. Electrophoretic evidence for a genetic admixture of native and non-native rainbow trout in the Yakima River, Washington. Trans. American Fish. Soc. 114:782-793.
- Campton, D.E., and F.M. Utter. 1985. Natural hybridization between steelhead trout (Salmo gairdneri) and coastal cutthroat trout (Salmo clarki clarki) in two Puget Sound streams. Can. J. Fish. Aquat. Sci. 42:110-119.
- Campton, D.E., and F.M. Utter. 1987. Genetic structure of anadromous cutthroat trout (Salmo clarki clarki) populations in the Puget Sound area: Evidence for restricted gene flow. Can. J. Fish. Aquat. Sci. 44:573-582.
- Carl, G.C., W.A. Clemens, and C.C. Lindsey. 1959. Freshwater fishes of British Columbia. Handbook No. 5, Dept. of Education, British Columbia Provincial Museum.
- Cooper, E.L. 1970. Growth of cutthroat trout (Salmo clarki) in Chef Creek, Vancouver Island, British Columbia. J. Fish. Res. Board. Can. 27(11):2063-2070.
- Crawford, B.A. 1979. The origin and history of the trout brood stocks of the Washington Department of Game. Fishery Research Report, Washington State Game Dept., Olympia, WA.

- Danzmann, R.G. and N.E. Down. 1982. Isozyme expression in F₁ hybrids between carp and goldfish. *Biochem. Genet.* 20:1-15.
- Dewitt, J.W. 1954. A survey of the coastal cutthroat trout, Salmo clarki clarki Richardson, in California. *Calif. Fish and Game* 40:329-335.
- Dimick, R.E. and F. Meryfield. 1945. The fishes of the Willamette River system in relation to population. *Oregon State Univ., Eng. Exp. Station Bull. No. 20*: 58pp.
- Donaldson, L.R., D.D. Hansler, and T.N. Buckridge. 1957. Interracial hybridization of cutthroat trout, Salmo clarki, and its use in fisheries management. *Trans. Amer. Fish. Soc.*, Vol. 86(1956):350-360.
- Engel, w., P. Kuhl, and J. Schmidtke. 1977. Expression of the paternally derived phosphoglucose isomerase genes during hybrid trout development. *Comp. Biochem. Physiol.* 56B:103-108.
- Fournier, D.A., T.D. Beacham, B.E. Riddell, and C.A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. *Can. J. Fish. and Aquat. Sci.* 41:400-408.
- Goodfellow, W.L., Jr., C.H. Hocutt, R.P. Morgan II, and J. R. Stauffer, Jr. 1984. Biochemical assessment of the taxonomic status of Rhinichthys bowseri (Pisces: Cyprinidae). *Copeia* 1984:652-659.
- Grant, W.S., G.B. Milner, P. Krasnowski, and F.M. Utter. 1980. Use of biochemical genetic variants for identification of sockeye salmon (Oncorhynchus nerka) stocks in Cook Inlet, Alaska. *Can. J. Fish. Aquat. Sci.* 37:1236-1247.
- Haig-Brown, R.L. 1947. The western angler - an account of Pacific salmon and western trout in British Columbia. Wm. Morrow and Co., NY.
- Hart, J.L. 1973. Pacific fishes of Canada. *Fish. Res. Board. Can.*, Bull. No. 180:740PP.
- Hartman, G.F., and C.A. Gill. 1968. Distributions of steelhead and cutthroat trout (Salmo gairdneri and S. clarki clarki) within streams on southwestern British Columbia. *Can. J. Fish. Aquat. Sci.* 25:33-48.

- Hitzeroth, H., J. Klose, S. Ohno, and U. Wolf. 1968. Asynchronous activation of parental alleles at the tissue-specific gene loci observed on hybrid trout during early development. *Biochem. Genet.* 1:287-300.
- Hoar, W.S. 1976. Smolt transformation: Evolution, behavior, and physiology. *J. Fish. Res. Board Can.* 33:1233-1252.
- Ihssen, P.E., H.E. Booke, J.M. Casselman, J.M. McGlade, N.R. Payne, and F.M. Utter. 1981. Stock identification: Materials and Methods. *Can. J. Fish. Aquat. Sci.* 38:1838-1855.
- Iwatsubo, T.T. and R.C. Averett. 1981. Aquatic biology of the Redwood Creek and Mill Creek drainage basins, Redwood National Park, Humboldt and Del Norte counties, California. U.S.G.S., Open-File Report 81-143. 115pp.
- Johnston, J.M. and S.P. Mercer. 1976. Sea-run cutthroat in salt water pens: broodstock development and extended juvenile rearing (with a life history compendium). Fishery Research Report, Project AFS-57-1, Washington State Game Dept., Olympia, WA.
- Larkin, P.A. 1981. A perspective on population genetics and salmon management. *Can. J. Fish. Aquat. Sci.* 38:1469-1475.
- Leary, R.F., F.W. Allendorf, S.R. Phelps, and K.L. Knudsen. 1984. Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. *Proceedings of the Montana Academy of Sciences* 43:1-18.
- Lestelle, L.C. 1978. The effects of forest debris removal on a population of resident cutthroat trout in a small headwater stream. M.S. Thesis, University of Washington, Seattle, WA. 86pp.
- McCleod, M.J., D.L. Wynes, and S.I. Guttman. 1980. Lack of biochemical evidence for hybridization between two species of darters. *Comparative Biochemistry and Physiology B* 67:323-325.
- Martin, F.D., and R.C. Richmond. 1973. An analysis of five enzyme gene loci in four theostomid species (Percidae:Pisces) in an area of possible introgression. *Journal of Fishery Biology* 5:511-517.

- May, B., J.E. Wright, and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with Salvelinus and a review of the literature on other species. J. Fish. Res. Board Can. 36:1114-1128.
- Meehan, W.R. 1974. The forest ecosystem of southeast Alaska. Part 3, Fish Habitats. U.S.D.A., Forest Service., Gen. Tech. Report, PNW-15-FR6, 41pp.
- Miller, R.R. 1950. Notes on the cutthroat and rainbow trouts with the description of a new species from the Gila River, New Mexico. Occas. Pap. Mus. Zool., Univ. Mich., No. 259, University of Michigan Press, Ann Arbor, MI. 42pp.
- Milner, G.B., D.J. Teel, F.M. Utter, and G.A. Winans. 1985. A genetic method of stock identification in mixed populations of Pacific salmon, Oncorhynchus spp. Marine Fisheries Review 47:1-8.
- Moring, J.R. and R.L. Lantz. 1975. The Alsea watershed study: Effects of logging on the aquatic resources of three headwater streams of the Alsea River, Oregon. Part I-Biological studies. Oregon Dept. Fish and Wild., Res. Sec., Fish. Res. Rept., No. 9:66pp.
- Moring, J.R. and R.M. Hooten. 1978. Oregon rainbow and cutthroat trout evaluation. Oregon Dept. Fish and Wild., Res. and Dev. sec., Prog. Rept., Fish., Proj. No. F-94-R-6:27pp.
- Neave, F. 1949. Game fish populations of the Cowichan River. Fish. Res. Board Can., Bull. No. 84:32pp.
- Needham, P.R., and R. Gard. 1959. Rainbow trout in Mexico and California with notes on the cutthroat series. Univ. Calif. Publ. Zool. 67:1-24.
- Nicholas, J.W. 1977. The feasibility of a consumptive wild trout fishery on the North Fork of the Middle Fork of the Willamette River. M.S. Thesis, Oregon State Univ., Corvallis, OR, 32pp.
- Nicholas, J.W. 1978a. A review of literature and unpublished information on cutthroat trout (Salmo clarki clarki) of the Willamette watershed. Oregon Dept. Fish and Wild., Res. Sec., Inform. Rept. Ser., Fish, No. 78-1:22pp.

- . 1978b. Life history differences between sympatric populations of rainbow and cutthroat trouts in relation to fisheries management strategy. Pages 181-188 in J. Moring (ed.). Proceedings of the Wild trout-catchable trout symposium. Oregon Dept. Fish and Wild., Res. and Dev. Sec., 210pp.
- Nyman, L. 1970. Electrophoretic analysis of hybrids between salmon (Salmo salar) and trout (Salmo trutta). Trans. Amer. Fish. Soc. 99:229-236.
- Ohno, S. 1969. The preferential activation of maternally derived alleles in development of interspecific hybrids. Wistar Symp. Monogr. 9:137-150.
- Pella, J.J. and G.B. Milner. 1985. Use of genetic marks in stock composition analysis. Pgs. 247-276. In N. Ryman and F. Utter (ed.) Population Genetics and Fishery Management. U. of Wash. Press, Seattle, WA, 420pp.
- Pelzmann, R.J. 1980. Impact of Florida largemouth bass Micropterus salmoides floridanus introductions at selected northern California waters, with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. California Fish and Game 66:133-162.
- Reinitz, G.L. 1977. Electrophoretic distinction of rainbow trout (Salmo gairdneri), west slope cutthroat trout (Salmo clarki), and their hybrids. J. Fish. Res. Board Can. 34:1236-1239.
- Reisenbichler, R.R. and S.R. Phelps. 1985. Genetic variation in chinook, Oncorhynchus tshawytscha, and coho, Oncorhynchus kisutch, salmon from the north coast of Washington. U.S. Fish and Wild. Fish Bull. 85(4):681-702.
- Royal, L.A. 1972. An examination of the anadromous trout program of the Washington State Game Department. Wash. State Game Dept., Final Rept., AFS-49:176pp.
- Scott, W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184:966pp.
- Shaw, C.R., and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Genet. 4:297-320.
- Sumner, F.H. 1948. The coastal cutthroat trout. Oregon State Game Comm., Bull. 3(12):1, 6-8.

- Utter, F.M., H.O. Hodgins, and F.W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations, p. 213-238. In D.C. Mulins and J.R. Sargent (ed.) Biochemical and biophysical perspectives in marine biology, Vol. 1. Academic Press, New York, NY.
- Utter, F.M., G.B. Milner, G. Stahl, and D. Teel. 1987. Genetic population structure of chinook salmon, Oncorhynchus tshawytscha, in the Pacific Northwest. U.S. Fish and Wild. Fish. Bull. 87(2):239-264.
- Whitmore, D.H. 1983. Interspecific hybridization of smallmouth and Guadalupe bass (Micropterus): Evidence based on biochemical genetic and morphological analyses. Southwestern Naturalist 27:99-106.
- Wishard, L.N., J.E. Seeb, F.M. Utter, and D. Stefan. 1984. A genetic investigation of suspected redband trout populations. Copeia 1984:120-132.
- Wyatt, B. 1959. Observations on the movements and reproduction of the Cascade form of cutthroat trout. M.S. Thesis, Oregon State Univ., Corvallis, 60pp.
- Wydoski, R.S. and R.R. Whitney. 1979. Inland fishes of Washington. University of Washington Press, Seattle, WA, and London. 220pp.