

AN ABSTRACT OF THE THESIS OF

Christian E. Zimmerman for the degree of Master of Science in Fisheries Science
presented on October 23, 1995. Title: Population Structure of Coastal Cutthroat Trout
(*Oncorhynchus clarki clarki*) in the Muck Creek Basin, Washington.

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Abstract approved: _____

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Gordon H. Reeves

The relationship of coastal cutthroat trout (*Oncorhynchus clarki clarki*) populations in the Muck Creek basin, a 238 km² southern Puget Sound stream basin in western Washington, was examined using starch gel electrophoresis and meristic analysis. Coastal cutthroat trout were collected from six sites throughout the basin including tributaries, portions of the mainstem, and a lake. Four sites contained only resident trout, the lower mainstem contained resident and anadromous trout, and the lake contained only mature anadromous trout based on size and appearance. Patterns of allelic and meristic variation suggest a significant structuring and separation of coastal cutthroat trout populations in the basin. The lake population was distinguished from the other populations by significant differences in allele frequencies and meristic characters. The other sites grouped more closely together with significant variation among and between sites meristically and at several loci.

Population Structure of Coastal Cutthroat Trout (*Oncorhynchus clarki clarki*)
in the Muck Creek Basin, Washington

By

Christian E. Zimmerman

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed October 23, 1995
Commencement June 1996

Master of Science thesis of Christian E. Zimmerman presented on October 23, 1995

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ACKNOWLEDGEMENT

I wish to acknowledge the help and advice provided by my committee: Dr. Gordon Reeves, Dr. Dede Olsen, and Dr. Hiram Li. Numerous people helped collect fish including: Kirstin Shumaker, Thomas Williams, Bill Gale, and the ALI summer field crew. Ken Currens provided advice and assistance that is greatly appreciated. Neil Ward orchestrated the electrophoresis. Numerous discussions with Ken Currens, Kitty Griswold, Neil Ward, and Thomas Williams helped form the ideas that became this thesis. Bruce Hansen helped negotiate a maze of purchase orders and travel authorizations. Thomas Williams provided the Bockette Creek data. Jim Stephenson, formerly of Fort Lewis, helped initiate this study and showed me how to find my way around heavy artillery impact zones and the Muck Creek basin. Funding was provided by the Department of Defense and the U.S. Forest Service.

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Population Structure of Coastal Cutthroat Trout (*Oncorhynchus clarki clarki*)
in the Muck Creek Basin, Washington

INTRODUCTION

Salmonids exhibit a variety of life history characteristics including anadromy and residency. Anadromous forms exploit relatively rich oceanic food resources, attain larger sizes feeding in the ocean, and have higher fecundity to offset the costs of migration. In resident forms the inherent benefits of large size may be reduced, and reproducing as early as possible (though at a smaller size) may be favorable (Stearns 1976). Several species of salmonids exhibit both resident and anadromous life history forms and the links between these two forms are poorly understood.

Do resident and migratory forms represent "ecophenotypes" within a single gene pool (Skaala and Nævdal 1989) or do they each represent genetically distinct populations? Some genetic control of anadromy or residency has been identified for a variety of species by several investigators (e.g., Jonsson 1982; Foote et al. 1989; Skaala and Nævdal 1989). Other studies, however, show that both genetic and environmental factors are involved in the control of resident or migratory behavior (e.g., Nordeng 1983; Jonsson 1985). Currens (1987) determined that resident rainbow trout (*Oncorhynchus mykiss*) in the Deschutes River, Oregon, may derive from either resident or anadromous forms although some isolation between these forms was detected. Northcote (1992) suggested that strong genetic control for residency is best developed in populations living in habitats where emigration could be disadvantageous (e.g., headwater streams, reaches above waterfalls or other barriers). Northcote et al. (1970) compared populations of rainbow trout above and below a waterfall using number of parr marks, scale rows, and vertebrae in addition to electrophoretic analyses. Both meristic and electrophoretic data indicated consistent differences between above and below falls populations, suggesting that the populations were genetically differentiated.

Genetic divergence of populations requires reproductive isolation. This can either be spatial or temporal separation in spawning activity. Baglinière et al. (1989) found spatial and some temporal separation of spawning between resident and migratory populations of brown trout (*Salmo trutta*) in a small stream in France. The resident population spawned only in the uppermost reaches of the system and the migratory population spawned primarily in the middle reaches. The two populations also spawned at different times during the study. Baglinière et al. (1989) concluded that spatial separation of spawning was the primary mechanism separating the two populations.

In general terms, a species that covers a wide range of aquatic environments by partitioning into resident and migratory forms may be in a better position for long-term survival where conditions are dynamic and unpredictable (Northcote 1992). Life history variation may, therefore, provides an important mechanism for preserving small populations of salmonids in marginal habitats. Saunders and Schom (1985) examined the high variability of life history parameters in Atlantic salmon (*Salmo salar*). Atlantic salmon exhibit variable ages at maturity and may spawn more than once. Saunders and Schom (1985) concluded that this variability in life histories may act as a safeguard against loss of small populations living in harsh conditions (i.e., small streams prone to large annual variations of discharge). As a result of the mixture of age classes in the spawning population, the effective spawning population may be potentially larger, and the level of inbreeding relatively low.

Coastal cutthroat trout (*O. clarki clarki*) reside in coastal streams from the Eel River in northern California to Prince William Sound in Alaska (Behnke 1992). Coastal cutthroat trout life history patterns include anadromous, potomadromous, lacustrine, and resident forms (Johnston 1981; Fuss 1982). Throughout their range there is a high degree of variation in spawning times, age at sexual maturity, growth, meristics, and age at smolting (Sumner 1953; Giger 1972; Johnston 1981; Behnke 1992). This variation in traits is evident in different life history forms.

Anadromous coastal cutthroat trout migrate to saltwater and return to their natal stream to spawn. Anadromous cutthroat trout often conduct annual migrations to saltwater after their initial migration, returning to freshwater each year to spawn or overwinter

(Sumner 1953; Armstrong 1971; Jones 1977; Johnston 1981). Sumner (1953) reported survival rates of 30% between the first and second spawning, 17% between the second and third spawning, and 12% between the third and fourth spawning in Sand Creek, Oregon. Anadromous coastal cutthroat trout are reported to have high fidelity to spawn in their natal stream (Johnston 1981; Campton and Utter 1987), although low levels of straying have been reported (Giger 1972).

Non-anadromous populations of coastal cutthroat trout exist as lacustrine and stream resident forms. Lacustrine forms migrate to lakes within the same basin to rear and return to their natal stream to spawn. Resident forms remain within their natal stream (usually small headwater streams), are usually smaller and less fecund, mature at an earlier age, and tend to spawn later in the season than their downstream counterparts (Northcote 1992). Campton and Utter (1987) suggested that resident populations are particularly sensitive to bottleneck effects and genetic drift. Also, they would be under a separate regime of natural selection since smolting and saltwater adaptation are not present as life history components.

Numerous investigators have speculated that headwater populations of resident cutthroat trout may contribute to the sea-run population (e.g., Jones 1977). The extent to which sympatric resident and anadromous coastal cutthroat trout interbreed is unknown. Campton and Utter (1987) identified biochemical differences between a nonanadromous population and anadromous populations of coastal cutthroat trout in northern Puget Sound. The nonanadromous population was characterized by low average heterozygosity, which would be expected from random genetic drift in a small reproductively isolated population. In tagging experiments of headwater resident populations of coastal cutthroat trout, Michael (1983) concluded that resident populations did not contribute to the anadromous population. The connection between sympatric resident and anadromous forms of coastal cutthroat trout has become a serious management consideration and needs further investigation (Johnson et al. 1994).

Intraspecific variation within basins has significant management implications to coastal cutthroat trout. Campton and Utter (1987) identified regional differences among populations of coastal cutthroat trout. Studies of Atlantic salmon suggest significant

genetic variation may exist among sections of a stream basin (e.g., Hurrel and Price 1993).

Land-use management is beginning to focus on the importance of small headwater streams and it is apparent that our knowledge of the fish which reside in such habitats is lacking. Resident populations of coastal cutthroat trout in small streams may represent important sources of genetic diversity which need protection and preservation. How we provide such protection will require information on the population structure of coastal cutthroat trout in small streams.

In this study, I used electrophoretic and meristic methods to investigate the relationship of coastal cutthroat trout populations in the Muck Creek watershed, Fort Lewis, Washington. Allele frequencies and meristic characters were compared among locations within the basin. A primary objective of this study was to determine the amount of variation and differentiation possible in a small stream basin without geologic barriers to migration such as waterfalls. This study was based on the hypothesis that coastal cutthroat trout populations collected from different sections of the basin would represent isolated populations as inferred through genetic and meristic analysis.

METHODS

Study Area

Muck Creek is a third order stream located in the southern Puget Sound region of western Washington (Figure 1). As a tributary to the Nisqually River, entering at river km 17, the Muck Creek watershed encompasses 238 km². The drainage has been moderately developed with rural residential homes, farms, and pastureland. Undeveloped portions consist of second growth coniferous forest, deciduous forest, and prairie (Williams et al. 1975). Most of the lower 11 km of Muck Creek lies within the Fort Lewis Military Reservation. The stream is low gradient and consists primarily of pool and glide habitats, small lakes, and several marshy areas distributed throughout the drainage. Chambers Lake is a natural 40.5 ha lake. A small dam with a fish ladder serves to increase the size of Chambers Lake. There are no geologic barriers to migration, such as waterfalls, within the system, although, Muck Creek experiences low flow conditions during the late summer and portions of the stream are dry for a month or more each year. Significant portions of the stream between Chambers Lake and the 13th Division prairie, the confluence of North and South Muck Creeks, and the area just downstream of the Chambers Lake dam are dry during this period.

Historically, the Muck Creek system supported chum salmon (*O. keta*), coho salmon (*O. kisutch*), steelhead trout (*O. mykiss*), and cutthroat trout (Williams et al. 1975). Numerous non-native species, including hatchery reared rainbow trout, brook trout (*Salvelinus fontinalis*), largemouth bass (*Micropterus salmoides*), sunfish (*Lepomis* spp.), black crappie (*Pomoxis nigromaculatus*), and yellow perch (*Perca flavescens*), have been introduced to the basin at Chambers Lake and Johnson Marsh. The system is currently known to support cutthroat trout, chum salmon, coho salmon, largemouth bass, sunfish, yellow perch, and threespine stickleback (*Gasterosteus aculeatus*).

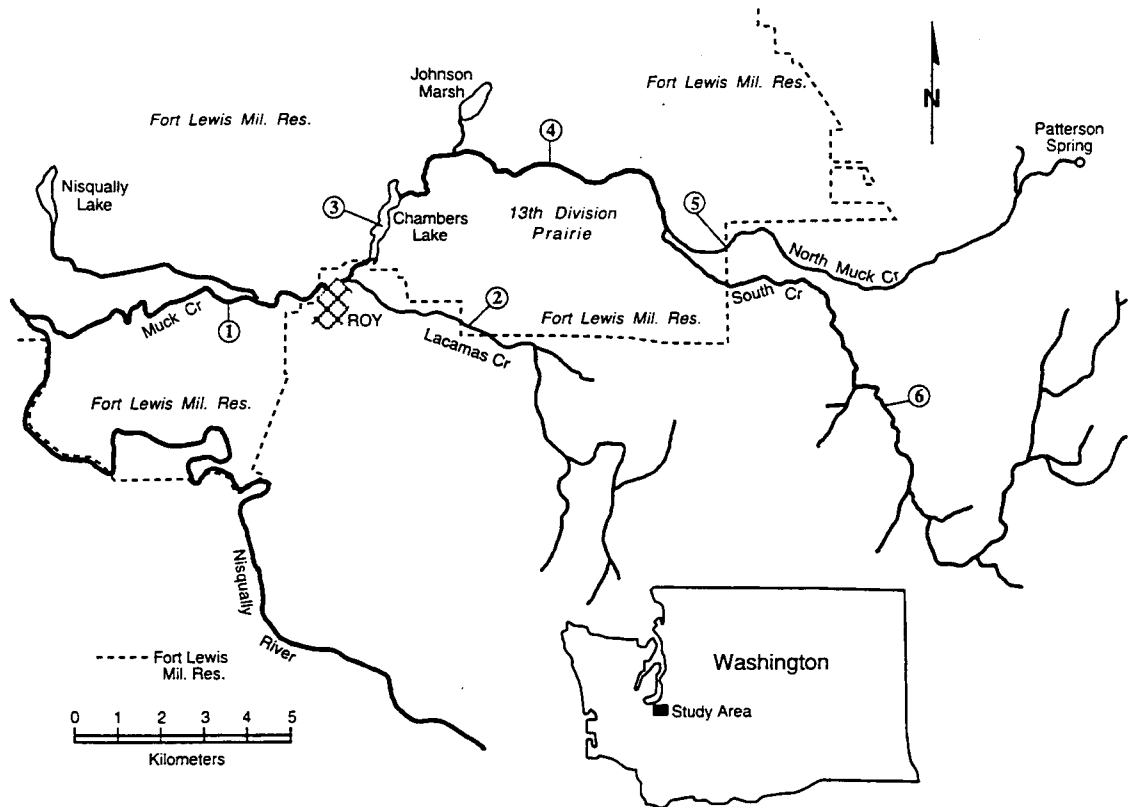


Figure 1. Study sites and location of Muck Creek, Fort Lewis Military Reservation, Washington, Study sites are: 1 = Muck Creek below Chambers Lake, 2 = Lacamas Creek, 3 = Chambers Lake, 4 = 13th Division Prairie, 5 = North Muck Creek, 6 = South Muck Creek.

Collection of Fish

Coastal cutthroat trout were collected from six study sections throughout the Muck Creek basin (Figure 1). The six study sections were defined based on geographic location within the basin, summer dry barriers, and potential limits of anadromy. Muck Creek below Chambers Lake included the main stem Muck Creek from the Chambers Lake dam to the confluence with the Nisqually River and Halvorsen Marsh. Lacamas Creek included the entire Lacamas Creek drainage. Chambers Lake include all stillwater areas above the Chambers Lake dam and below the inlet of Muck Creek. The 13th Division Prairie included the main stem of Muck Creek between Chambers Lake and the confluence of North and South Muck Creeks. North Muck Creek and South Muck Creek included the main stem of each above their confluence. Nisqually Lake was excluded from the study because it lies within a heavy-artillery impact area and Johnson Marsh was excluded because only one coastal cutthroat was collected.

A backpack electrofisher was used to collect all fish from stream sections of Muck Creek and gill-nets were used to collect fish from Chambers Lake, Halvorsen Marsh, and Johnson Marsh. Collections ranged from 25 to 55 fish (Table 1). Although the timing of collections spanned the months of April through November, 91% of the fish were collected during the months of June through September. With the exception of Chambers Lake, all samples included both juveniles and mature adults. All Chambers Lake fish were sexually mature. Each fish was weighed, measured (fork length), and tagged with a unique identifying number before being frozen on dry ice until transferred to a -80°C freezer. Tissues, which included eye, muscle, liver, and heart, were extracted in the field and frozen separately from fish that were too large (>250 mm fork length) to be frozen quickly by the dry ice.

Electrophoresis

Methods of electrophoresis were those of Aebersold et al. (1987) and were conducted at the Oregon Cooperative Fishery Research Unit at Oregon State University. Enzyme and locus nomenclature (Table 2) followed Shaklee et al. (1990). The frequency

Table 1. Number collected, mean fork length (standard deviation), and dates collections made of coastal cutthroat trout from the Muck Creek basin, Washington.

<u>Location</u>	<u>Number Collected</u>	<u>Mean Fork Length (mm)</u>	<u>Dates Collected</u>
Muck Creek Below Chambers Lake	48	158.33 (78.37)	18 November 1992 8 July 1993 12 August 1993 26 August 1993 7 September 1993
Lacamas Creek	33	147.89 (39.22)	8 September 1994 22 September 1994 9 April 1994
Chambers Lake	25	300.48 (63.62)	21 June 1993 8 July 1993 16 June 1994
13th Division Prairie	55	138.26 (53.58)	18 November 1992 7 July 1993 21 September 1993
North Muck Creek	40	133.55 (41.68)	8 September 1993 9 April 1994
South Muck Creek	25	105.48 (30.79)	22 September 1993

Table 2. International Union of Biochemistry (I.U.B.) enzyme names, Enzyme Commission (E.C.) numbers, loci examined in this study, tissues, and buffers. Tissues are: muscle (M), liver (L), eye (E), and heart (H).

I.U.B Enzyme Name	E.C. Number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	<i>sAAT-1,2*</i>	M	TBCLE
Alcohol dehydrogenase	1.1.1.1	<i>ADH*</i>	L	ACE
Aconititate hydratase	4.2.1.3	<i>sAH*</i>	L	ACE
		<i>mAH-3*</i>	H	ACE+N
		<i>mAH-4*</i>	H	ACE
Adenylate kinase	2.7.4.3	<i>AK-1*</i>	M	ACE
		<i>AK-2*</i>	M	ACE
Creatine kinase	2.7.3.2	<i>CK-A1*</i>	M	TBCLE
		<i>CK-A2*</i>	M	TBCLE
		<i>CK-B*</i>	E	TG/KG
		<i>CK-C1*</i>	E	TG/KG
		<i>CK-C2*</i>	E	TG/KG
Fructose-bisphosphate aldolase	4.1.2.13	<i>FBALD-1*</i>	E	TG/KG
		<i>FBALD-2*</i>	E	TG/KG
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	<i>GAPDH-1*</i>	E	ACE
		<i>GAPDH-2*</i>	E	ACE
		<i>GAPDH-3*</i>	E	ACE
		<i>GAPDH-4*</i>	E	ACE
		<i>GAPDH-5*</i>	E	ACE
Guanine deaminase	3.5.4.3	<i>GDA-1*</i>	L	TBCLE
		<i>GDA-2*</i>	L	TBCLE
Glycerol-3-phosphate dehydrogenase	1.1.1.8	<i>G3PDH-1*</i>	M	ACE
		<i>G3PDH-2*</i>	M	ACE
Glucose-6-phosphate isomerase	5.3.1.9	<i>GPI-A*</i>	M	TG/KG
		<i>GPI-B1*</i>	M	TBCLE
		<i>GPI-B2*</i>	M	TBCLE

Table 2 (continued).

I.U.B Enzyme Name	E.C. Number	Locus	Tissue	Buffer
Glutathione reductase	1.6.4.2	<i>GR*</i>	H	ACE+N
Isocitrate dehydrogenase (NADP ⁺)	1.1.1.42	<i>mIDHP-1*</i>	M	ACE
		<i>mIDHP-2*</i>	M	ACE
		<i>sIDHP-1,2*</i>	L	ACE
L-lactate dehydrogenase	1.1.1.27	<i>LDH-A1*</i>	M	TBCLE
		<i>LDH-A2*</i>	M	TBCLE
		<i>LDH-B1*</i>	E	TG/KG
		<i>LDH-B2*</i>	L	TBCLE
		<i>LDH-C1*</i>	E	TG/KG
Malate dehydrogenase	1.1.1.37	<i>sMDH-A*</i>	L	ACE
		<i>sMDH-B*</i>	H	ACE+N
		<i>mMDH-1*</i>	L	ACE
		<i>mMDH-2*</i>	H	ACE+N
Malate dehydrogenase (NADP ⁺)	1.1.1.40	<i>sMEP-2*</i>	L	ACE
Mannose-6-phosphate isomerase	5.3.1.8	<i>MPI*</i>	H	TBE
Dipeptidase	3.4.-.-	<i>PEP-A*</i>	M	TG/KG
Tripeptide aminopeptidase	3.4.-.-	<i>PEP-B*</i>	M	TG/KG
Proline dipeptidase	3.4.-.-	<i>PEP-P*</i>	M	ACE
Phosphoglucuronate dehydrogenase	1.1.1.44	<i>PGDH*</i>	M	ACE
Phosphoglucomutase	5.4.2.2	<i>PGM-1*</i>	M	ACE
		<i>PGM-2*</i>	M	ACE
Superoxide dismutase	1.15.1.1	<i>SOD*</i>	L	TBCLE

Table 2 (continued).

I.U.B Enzyme Name	E.C. Number	Locus	Tissue	Buffer
Triose-phosphate isomerase	5.3.1.1	<i>TPI-1*</i>	E	TG/KG
		<i>TPI-2*</i>	E	TG/KG
		<i>TPI-3*</i>	E	TG/KG
		<i>TPI-4*</i>	E	TG/KG

of genotypes at each locus was tested for departures from the assumptions of random mating using chi-square tests. Duplicated loci and loci with expected allele occurrences less than one were excluded from this analysis. Average heterozygosity, number of polymorphic loci, and the number of rare alleles (< 5.0 occurrence) were calculated for each sample site as measures of genetic variation within samples. Average heterozygosity was calculated for each locus using Hardy-Weinberg expectations and averaged over all loci. Pairwise chi-square tests were used to test hypotheses of allelic homogeneity among and between sampling sites. Only loci with mean frequencies of the common allele of less than 0.95 were used in the analysis. A dendrogram was constructed from a matrix of genetic distances (D)(Nei 1978) with the unweighted pair group method of arithmetic averages (UPGMA) to graphically represent genetic relationships among the sample sites. A second dendrogram of genetic distance was constructed that included data from 25 coastal cutthroat trout collected from Bockette Creek (Thomas Williams, Oregon State University, unpublished data), a small tributary of the Solduc River on the west side of the Olympic peninsula, as an outgroup to provide scale when examining the differences among the Muck Creek sites. The Bockette Creek data included 37 loci.

Meristics

After extraction of tissues for electrophoresis, all fish were preserved in 10% buffered formalin and stored in 70% ethyl alcohol. Meristic characters were defined according to Hubbs and Lagler (1964) and Behnke (1992). Meristic characters measured were: 1) scales in lateral line series, 2) scales above lateral line, 3) dorsal fin rays, 4) pectoral fin rays, 5) pelvic fin rays, 6) anal fin rays, 7) gill rakers, 8) branchiostegal rays, and 9) vertebrae. In all cases, counts were made on the left side of the fish. Gill rakers were stained with alizarin red one day prior to counting and pectoral and pelvic fin rays were stained with alizarin red just prior to counting. Mucus was gently scraped from the surface of the fish and scales were stained with malachite green just prior to counting. Counts of vertebrae, dorsal fin pterygiophores, and anal fin pterygiophores were made on x-ray radiographs.

One-way analysis of variance was used to determine the significance of differences in mean counts among sampling locations for each meristic character. Mean values and 95% confidence intervals were plotted to discern geographic patterns. Stepwise discriminant function analysis was performed to distinguish the structure of populations within the basin based on meristic variation.

RESULTS

Genetic Variation Within Samples

Of the 52 loci screened, 14 were polymorphic at the 0.95 criterion. Allele frequencies are presented in Table 3. Deviation from Hardy-Weinberg equilibrium was observed at the *PEP-B** locus in the South Muck Creek sample with a deficiency of heterozygotes. Otherwise, genotypic distribution conformed to that expected under Hardy-Weinberg equilibrium suggesting that each sample consisted of fish drawn from a single, randomly mating unit. Seventy-three loci were tested for deviation from Hardy-Weinberg equilibrium. The P-value of the South Muck Creek *PEP-B** test was equal to 0.05 and could represent a Type I error because 3 of the 73 tests are expected to result in P-values less than 0.05 by chance only. Given this lack of deviation from Hardy-Weinberg Equilibrium, I concluded that each sample could be treated as a separate population.

Average heterozygosity (H) ranged from 0.042 to 0.057 (Table 4). The number of rare alleles observed in each sample ranged from 4 to 9 (Table 4). There was no apparent pattern of distribution of H or rare alleles.

Genetic Variation Among Samples

Significant allele frequency differences were observed between the Chambers Lake sample and all other sites. Pair-wise comparisons of allele frequencies between populations demonstrated consistent differences between Chambers Lake and the other samples at the *ADH**, *GPI-B1**, *GDA-1**, and *GDA-2** loci (Table 5 and 6). Other loci demonstrated significant differences between sites, such as AK-1 which separated Lacamas Creek from the other sites (Table 7) and *PEP-A** which separated North Muck Creek from the other sites with the exception of South Muck Creek (Table 8). North Muck Creek and the 13th Division Prairie were significantly different from the Muck Creek below Chambers Lake and Lacamas Creek samples at the *PEP-B** locus (Table 7). Significant

Table 3. Isozyme frequencies and allele sample sizes for polymorphic loci in coastal cutthroat trout, Muck Creek basin, Washington.

Locus	Muck CreekBelow Chambers Lake	Lacamas Creek	Chambers Lake	13th Div. Prairie	North Muck Creek	South Muck Creek
<i>sAAT-1,2*</i>						
N	42	33	25	52	39	22
100	0.720	0.674	0.680	0.688	0.686	0.625
88	0.280	0.326	0.310	0.313	0.314	0.375
110	0.000	0.000	0.010	0.000	0.000	0.000
<i>ADH*</i>						
N	47	27	25	30	27	24
100	0.947	0.907	0.880	0.967	0.889	0.979
72	0.000	0.093	0.400	0.033	0.111	0.021
50	0.053	0.000	0.080	0.000	0.000	0.000
<i>sAH*</i>						
N	41	33	20	51	12	25
100	0.976	1.000	1.000	1.000	1.000	1.000
80	0.024	0.000	0.000	0.000	0.000	0.000
<i>mAH-3*</i>						
N	31	33	20	55	33	25
100	1.000	0.985	1.000	1.000	1.000	1.000
83	0.000	0.015	0.000	0.000	0.000	0.000
<i>mAH-4*</i>						
N	18	33	15	55	33	25
100	1.000	0.879	0.900	0.945	0.970	0.940
83	0.000	0.121	0.100	0.055	0.030	0.060
<i>AK-1*</i>						
N	48	33	25	55	40	25
100	1.000	0.909	1.000	1.000	1.000	0.960
20	0.000	0.091	0.000	0.000	0.000	0.040

Table 3 (continued).

Locus	Muck Creek Below Chambers Lake	Lacamas Creek	Chambers Lake	13th Div. Prairie	North Muck Creek	South Muck Creek
<i>AK-2*</i>						
N	48	33	25	55	40	25
100	1.000	0.985	1.000	1.000	1.000	0.960
65	0.000	0.015	0.000	0.000	0.000	0.040
<i>CK-A1*</i>						
N	48	33	25	55	40	25
100	1.000	1.000	0.960	1.000	1.000	1.000
88	0.000	0.000	0.040	0.000	0.000	0.000
<i>CK-A2*</i>						
N	48	33	25	55	40	25
100	1.000	0.985	1.000	1.000	1.000	1.000
112	0.000	0.015	0.000	0.000	0.000	0.000
<i>GDA-1*</i>						
N	46	32	25	55	40	21
100	0.913	0.797	1.000	0.845	0.813	0.762
115	0.087	0.203	0.000	0.155	0.188	0.238
<i>GDA-2*</i>						
N	46	33	24	55	40	22
100	0.935	0.985	0.396	0.891	0.975	1.000
87	0.065	0.015	0.604	0.109	0.025	0.000
<i>GPI-B1*</i>						
N	36	28	19	47	30	25
100	0.347	0.393	0.421	0.479	0.350	0.240
160	0.486	0.429	0.132	0.415	0.467	0.400
42	0.167	0.179	0.447	0.128	0.183	0.360

Table 3 (continued).

Locus	Muck Creek Below Chambers Lake	Lacamas Creek	Chambers Lake	13th Div. Prairie	North Muck Creek	South Muck Creek
<i>GPI-B2*</i>						
N	41	33	20	55	33	25
100	1.000	0.985	1.000	1.000	1.000	1.000
140	0.000	0.015	0.000	0.000	0.000	0.000
<i>sIDHP-1*</i>						
N	27	33	21	54	40	23
100	0.213	0.167	0.214	0.204	0.188	0.207
70	0.620	0.621	0.560	0.708	0.637	0.554
33	0.056	0.000	0.226	0.088	0.175	0.022
26	0.111	0.212	0.000	0.000	0.000	0.217
<i>LDH-A2*</i>						
N	48	33	25	55	40	25
100	1.000	1.000	1.000	0.991	1.000	1.000
87	0.000	0.000	0.000	0.009	0.000	0.000
<i>LDH-B1*</i>						
N	48	33	25	55	40	25
100	1.000	1.000	1.000	1.000	0.988	1.000
135	0.000	0.000	0.000	0.000	0.012	0.000
<i>LDH-B2*</i>						
N	48	33	25	55	40	25
100	1.000	1.000	1.000	0.991	1.000	1.000
76	0.000	0.000	0.000	0.009	0.000	0.000
<i>MDH-1*</i>						
N	48	33	25	55	20	25
100	0.927	0.955	0.880	0.873	0.900	0.870
115	0.073	0.045	0.110	0.114	0.087	0.130
83	0.000	0.000	0.010	0.014	0.013	0.000

Table 3 (continued).

Locus	Muck Creek Below Chambers Lake	Lacamas Creek	Chambers Lake	13th Div. Prairie	North Muck Creek	South Muck Creek
<i>MDH-2*</i>						
N	48	33	25	55	33	25
100	1.000	1.000	0.980	1.000	1.000	1.000
65	0.000	0.000	0.020	0.000	0.000	0.000
<i>sMEP-2*</i>						
N	48	33	20	55	33	25
100	0.000	0.000	0.000	0.027	0.000	0.000
119	1.000	1.000	1.000	0.973	1.000	1.000
<i>PEP-A*</i>						
N	48	33	25	55	40	25
100	0.031	0.091	0.020	0.036	0.112	0.100
112	0.958	0.879	0.980	0.964	0.788	0.900
123	0.010	0.015	0.000	0.000	0.100	0.000
75	0.000	0.015	0.000	0.000	0.000	0.000
<i>PEP-B*</i>						
N	47	33	25	51	38	23
100	0.436	0.409	0.540	0.627	0.658	0.543
69	0.564	0.591	0.460	0.373	0.342	0.457
<i>PEP-P*</i>						
N	41	33	20	55	33	25
100	1.000	1.000	0.950	0.964	1.000	1.000
110	0.000	0.000	0.050	0.036	0.000	0.000
<i>PGM-1*</i>						
N	35	32	19	50	39	25
100	0.900	0.891	0.868	0.910	0.936	0.940
84	0.100	0.109	0.132	0.090	0.064	0.060

Table 3 (continued).

Locus	Muck Creek Below Chambers Lake	Lacamas Creek	Chambers Lake	13th Div. Prairie	North Muck Creek	South Muck Creek
<i>PGM-2*</i>						
N	44	33	24	53	40	25
-100	0.886	0.879	0.813	0.811	0.837	0.860
-140	0.114	0.121	0.188	0.189	0.162	0.140
<i>SOD*</i>						
N	48	33	25	55	40	25
100	0.969	0.985	0.980	0.964	0.975	1.000
140	0.031	0.015	0.020	0.036	0.025	0.000
<i>TPI-4*</i>						
N	48	33	25	55	40	25
100	1.000	1.000	1.000	0.982	1.000	1.000
96	0.000	0.000	0.000	0.018	0.000	0.000

Table 4. Average heterozygosity, number of rare alleles, and number of loci with frequencies of the common allele greater than 0.95.

Site	Average Heterozygosity	Number of Rare Alleles	Number of Loci with $P > 0.95$
Muck Creek Below Chambers Lake	0.042	4	41
Lacamas Creek	0.057	9	40
Chambers Lake	0.057	6	42
13th Division Prairie	0.051	9	41
North Muck Creek	0.051	5	41
South Muck Creek	0.047	4	41

Table 5. Significance (P-value) of pair-wise contingency chi square tests of allelic homogeneity, *ADH** above diagonal and *GPI-B1** below diagonal, of coastal cutthroat trout, Muck Creek basin, Washington (*=P>0.05, **=P>0.001, n.s.=not significant).

Sample ¹	1	2	3	4	5	6
1	----	**	**	*	**	n.s.
2	n.s.	----	**	n.s.	n.s.	n.s.
3	**	**	----	**	**	**
4	n.s.	n.s.	**	----	n.s.	n.s.
5	n.s.	n.s.	**	n.s.	----	n.s.
6	n.s.	n.s.	*	**	n.s.	----

¹ 1=Muck Creek below Chambers Lake, 2=Lacamas Creek, 3=Chambers Lake, 4=13th Division Prairie, 5=North Muck Creek, 6=South Muck Creek

Table 6. Significance (P-value) of pair-wise contingency chi square tests of allelic homogeneity, *GDA-1** above diagonal and *GDA-2** below diagonal, of coastal cutthroat trout, Muck Creek basin, Washington (*=P>0.05, **=P>0.001, n.s.= not significant).

Sample ¹	1	2	3	4	5	6
1	----	*	*	n.s.	n.s.	n.s.
2	n.s.	----	**	n.s.	n.s.	n.s.
3	**	**	----	**	**	**
4	n.s.	*	**	----	n.s.	n.s.
5	n.s.	n.s.	**	*	----	n.s.
6	n.s.	n.s.	**	*	n.s.	----

¹ 1=Muck Creek below Chambers Lake, 2=Lacamas Creek, 3=Chambers Lake, 4=13th Division Prairie, 5=North Muck Creek, 6=South Muck Creek

Table 7. Significance (P-value) of pair-wise contingency chi square tests of allelic homogeneity, *AK-1** above diagonal and *PEP-B** below diagonal, of coastal cutthroat trout, Muck Creek basin, Washington (*=P>0.05, **=P>0.001, n.s.= not significant).

Sample ¹	1	2	3	4	5	6
1	----	**	n.s.	n.s.	n.s.	*
2	n.s.	----	*	**	**	n.s.
3	n.s.	n.s.	----	n.s.	n.s.	n.s.
4	**	**	n.s.	----	n.s.	*
5	**	**	n.s.	n.s.	----	n.s.
6	n.s.	n.s.	n.s.	n.s.	n.s.	----

¹ 1=Muck Creek below Chambers Lake, 2=Lacamas Creek, 3=Chambers Lake, 4=13th Division Prairie, 5=North Muck Creek, 6=South Muck Creek

Table 8. Significance (P-value) of pair-wise contingency chi square tests of allelic homogeneity, *PEP-A** above diagonal and *SIDHp-1,2** below diagonal, of coastal cutthroat trout, Muck Creek basin, Washington (*=P>0.05, **=P>0.001, n.s.= not significant).

Sample ¹	1	2	3	4	5	6
1	----	n.s.	n.s.	n.s.	**	n.s.
2	**	----	n.s.	**	**	n.s.
3	**	**	----	n.s.	**	n.s.
4	**	**	**	----	**	n.s.
5	**	**	n.s.	*	----	n.s.
6	n.s.	n.s.	**	**	**	----

¹ 1=Muck Creek below Chambers Lake, 2=Lacamas Creek, 3=Chambers Lake, 4=13th Division Prairie, 5=North Muck Creek, 6=South Muck Creek

allele frequency differences occurred throughout the basin at the *sIDHp-1,2** locus (Table 8). These relationships are further demonstrated by the dendrogram of Nei's genetic distance (Figure 2).

Meristics

One-way analysis of variance indicated that all meristic characters (Table 9) but two (vertebrae and pelvic fin rays) displayed significant differences among sampling sites (Table 10). Means plots indicated four distinct patterns of meristic variation within the basin. The first pattern, indicates a separation between sites above Chambers Lake and sites below and including Chambers Lake. This pattern was observed in lateral line series scales (Figure 3A) and dorsal fin pterygiophores (Figure 3B). The second distribution encountered was demonstrated by anal fin pterygiophores (Figure 4A) and pectoral fin ray counts (Figure 4B) where Chambers Lake separates from the other sample sites. The third pattern (Figure 5A), separated the 13th Division Prairie, Lacamas Creek, and North Muck Creek populations from the other sites and was exhibited by gill raker counts. The fourth pattern, (Figure 5B), separated Lacamas Creek from the other sites and was exhibited by branchiostegal ray counts.

Stepwise discriminant analysis of meristic characters indicated that all eight characters contributed significantly to group separation (Figure 6). One-way analysis of variance indicated significant differences among sampling sites for the first discriminant function ($F_{5,200} = 13.782, p < 0.0001$) and the second discriminant function ($F_{5,200} = 6.356, p < 0.0001$). Significant differences between sampling sites were evident in pairwise t-tests (Table 11). The number of scales above the lateral line count was excluded from the discriminant analysis because it was highly correlated with scales in the lateral line series ($r = 0.6255, p < 0.0001$). The first discriminant function, accounted for 50.86% of the among location variation and separated the upper and lower sites. The second discriminant function, accounted for 23.55% of the among location variation and significantly separated Chambers Lake and South Muck Creek from each other and the other 4 sites.

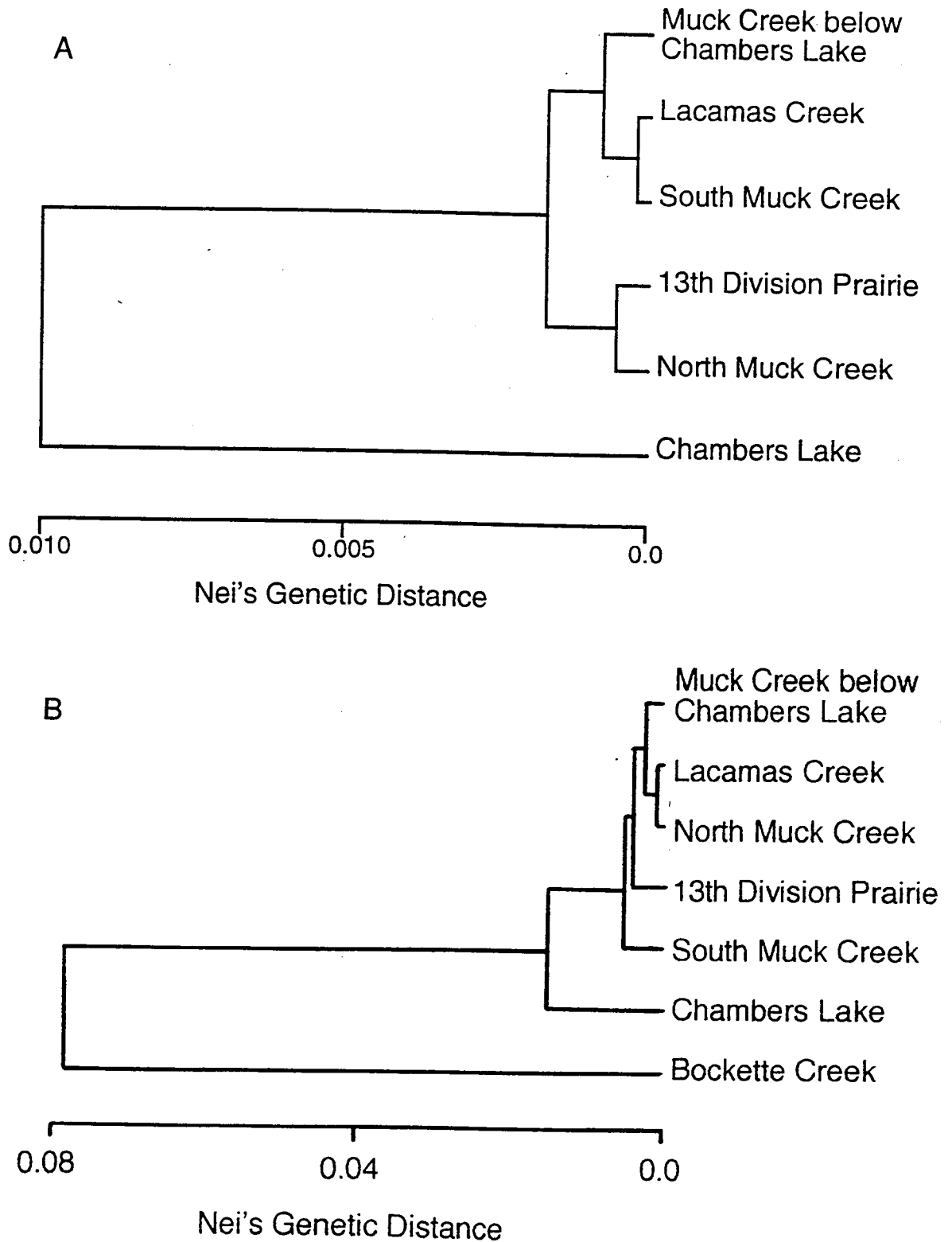


Figure 2. Dendrogram of genetic distance of coastal cutthroat trout populations in Muck Creek (A) and in Muck Creek with Bockette Creek as an outgroup (B). Not difference in scale between the dendrograms

Table 9. Means, standard errors in parentheses, and sample sizes for meristic characters of coastal cutthroat trout, Muck Creek, Washington.

Sample	N	Scales in Lateral Series	Scales Above Lateral Line	Dorsal Fin Pterygiophores	Anal Fin Pterygiophores	Pectoral Fin Rays	Pelvic Fin Rays
Muck Cr. Below Chambers Lake	48	143.94 (1.32)	32.50 (0.33)	10.92 (0.97)	11.13 (0.11)	12.98 (0.06)	8.98 (0.04)
Lacamas Creek	35	140.11 (1.71)	32.71 (0.49)	10.97 (0.12)	11.31 (0.11)	13.00 (0.12)	8.97 (0.06)
Chambers Lake	25	144.00 (1.67)	32.64 (0.34)	11.12 (0.11)	11.72 (0.11)	12.64 (0.11)	9.00 (0.06)
13th Division Prairie	55	134.87 (1.54)	30.71 (0.28)	10.67 (0.09)	11.02 (0.12)	12.78 (0.08)	8.91 (0.04)
North Muck Creek	40	136.45 (1.24)	30.98 (0.32)	10.77 (0.10)	11.20 (0.11)	13.00 (0.07)	8.95 (0.03)
South Muck Creek	25	132.52 (1.17)	30.72 (0.48)	10.71 (0.09)	11.00 (0.11)	13.08 (0.13)	9.00 (0.00)
Combined	228	138.93 (0.66)	31.57 (0.16)	10.84 (0.04)	11.21 (0.05)	12.91 (0.04)	8.96 (0.02)

Table 9 (continued).

Sample	Gill Rakers	Vertebrae	Branchiostegal Rays
Muck Creek Below Chambers Lake	17.94 (0.17)	60.83 (0.12)	10.81 (0.09)
Lacamas Creek	17.14 (0.18)	60.97 (0.16)	10.43 (0.11)
Chambers Lake	17.64 (0.24)	61.04 (0.16)	10.72 (0.14)
13th Division Prairie	16.94 (0.12)	61.15 (0.11)	11.06 (0.10)
North Muck Creek	17.05 (0.13)	61.33 (0.11)	10.80 (0.10)
South Muck Creek	17.92 (0.25)	61.20 (0.18)	10.68 (0.14)
Combined	17.39 (0.07)	61.08 (0.06)	10.78 (0.05)

Table 10. One-way analysis of variance F-statistics, degrees of freedom, and significance value of meristic characters of coastal cutthroat trout from different locations in the Muck Creek basin, Washington.

Meristic Character	F-ratio	Degrees of Freedom	P
Number of Scales in Lateral Series	7.648	5, 222	0.0000
Scales Above Lateral Line	6.121	5, 222	0.0000
Dorsal Fin Pterygiophores	2.758	5, 213	0.0195
Anal Fin Pterygiophores	4.042	5, 203	0.0016
Pectoral Fin Rays	2.719	5, 222	0.0209
Pelvic Fin Rays	0.673	5, 222	N.S.
Gill Rakers	7.430	5, 219	0.0000
Vertebrae	1.810	5, 222	N.S.
Branchiostegal Rays	3.959	5, 220	0.0019

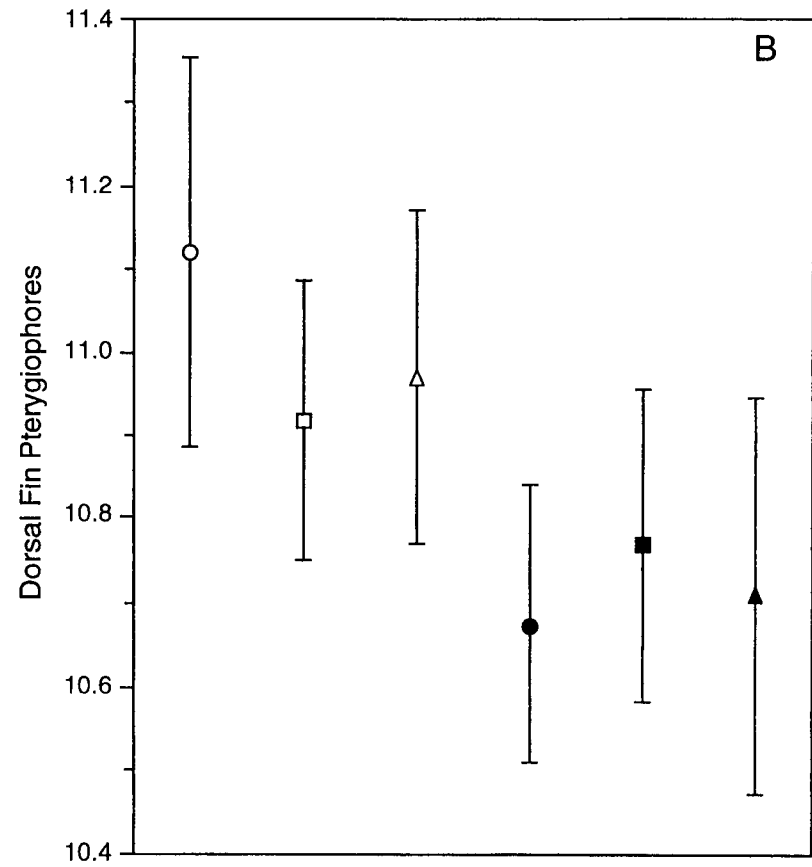
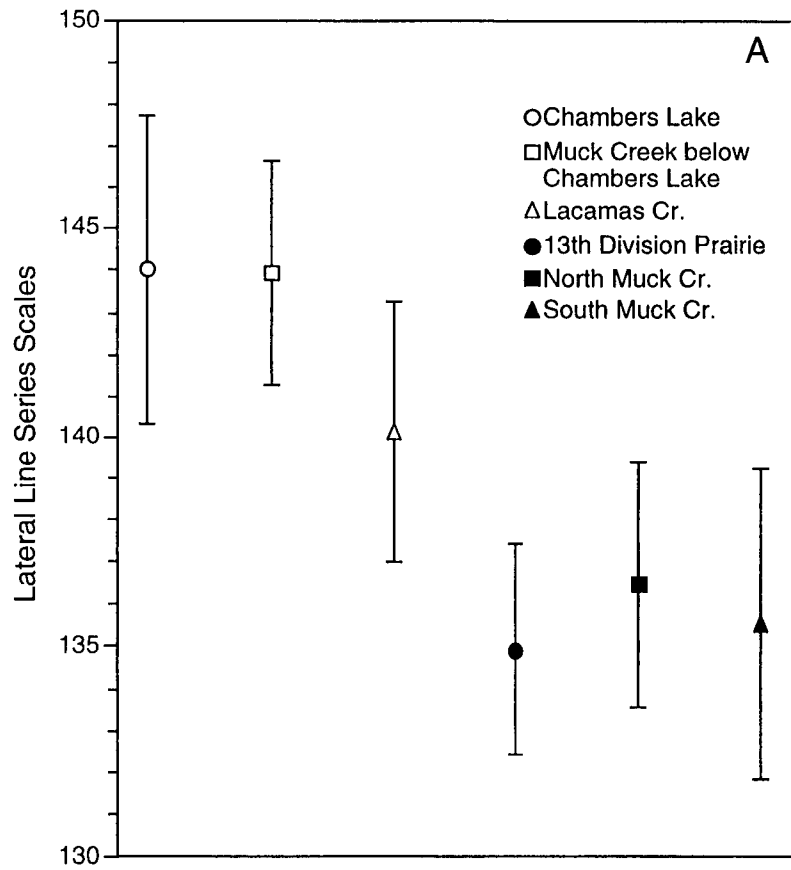


Figure 3. Means and 95% confidence intervals of lateral line series scale (A) and dorsal fin pterygiophore (B) counts of coastal cutthroat trout in the Muck Creek Basin, Washington.

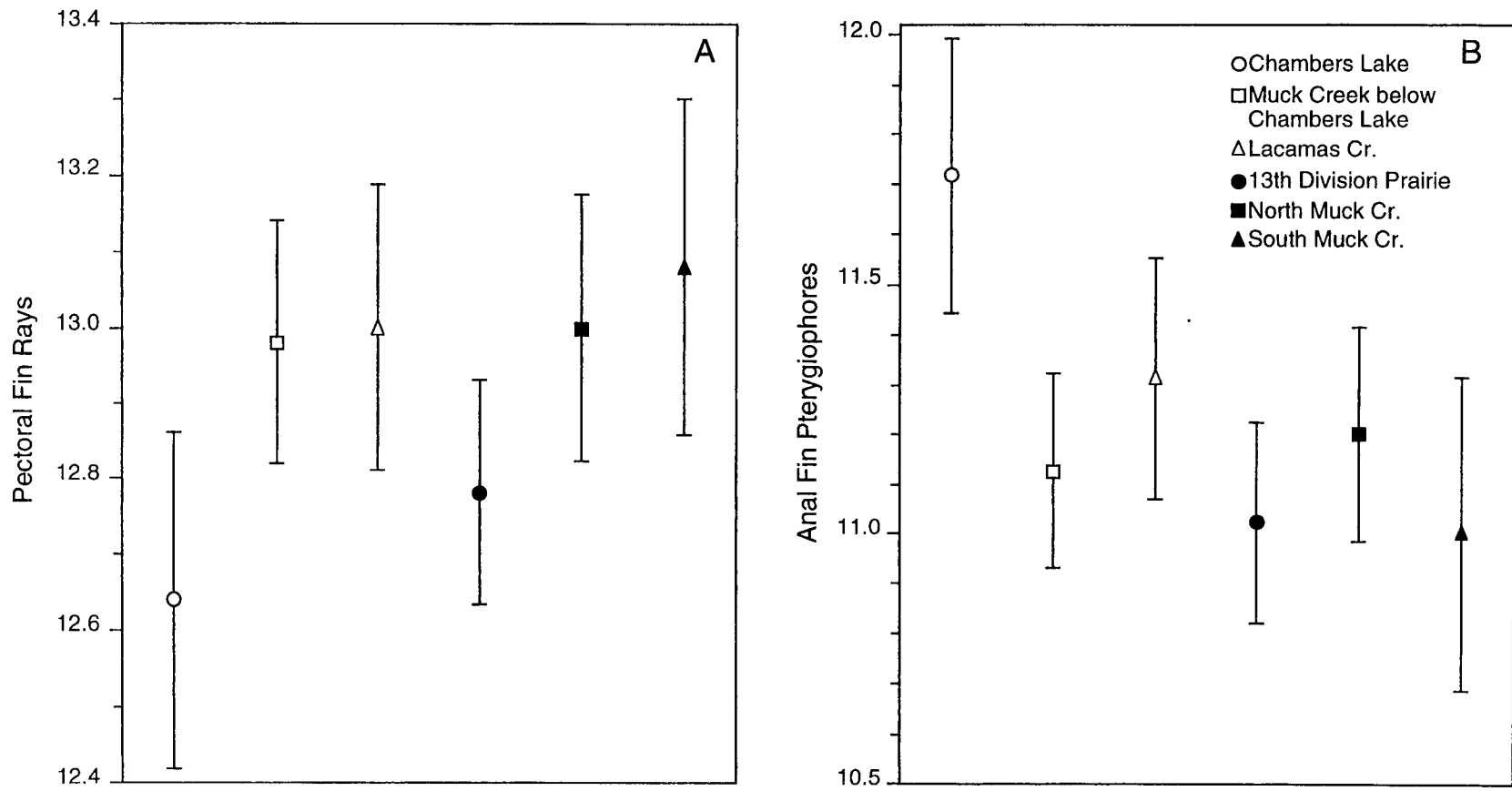


Figure 4. Means and 95% confidence intervals of pectoral fin ray (A) and anal fin pterygiophore (B) counts of coastal cutthroat trout in the Muck Creek Basin, Washington.

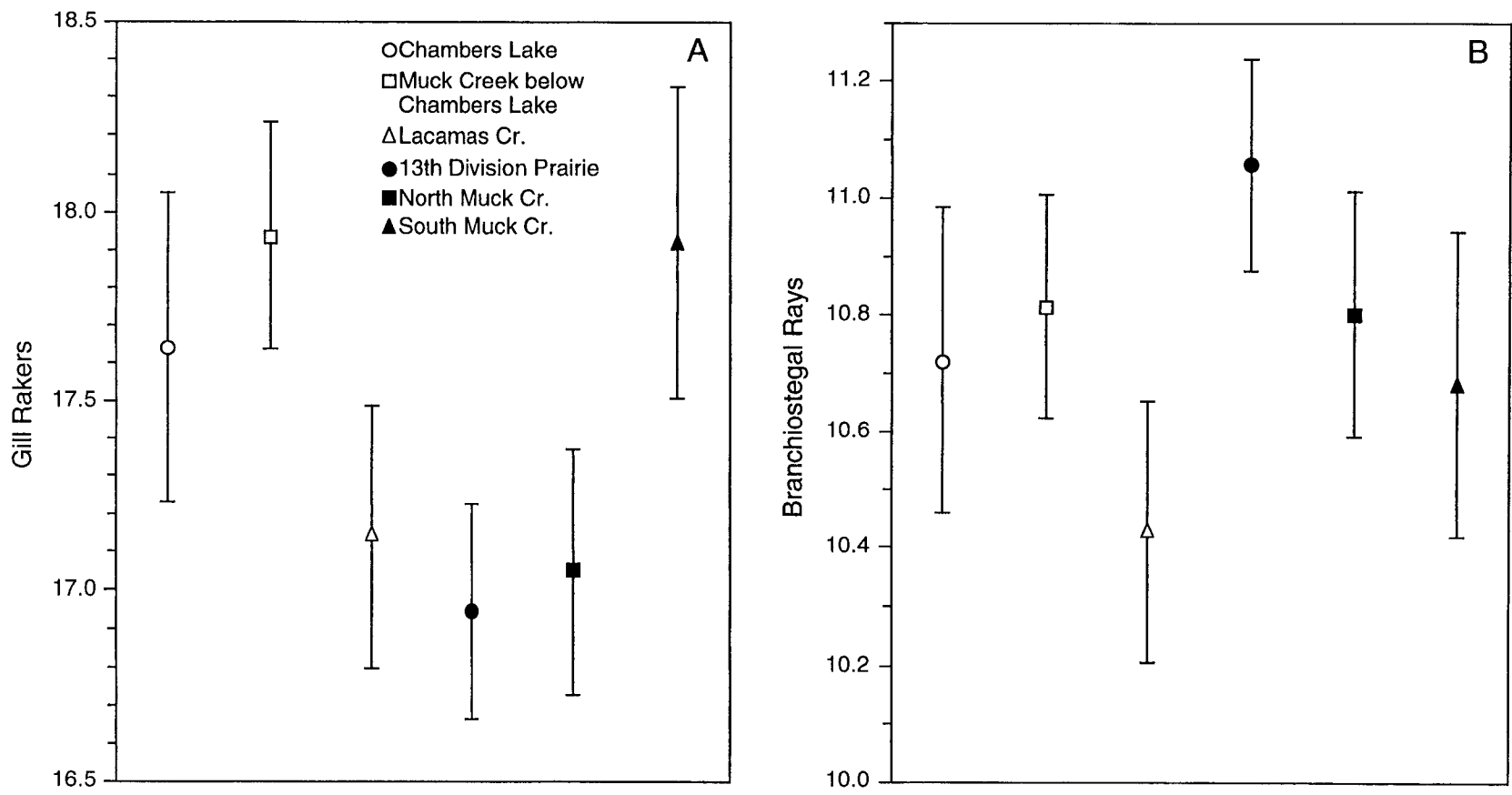
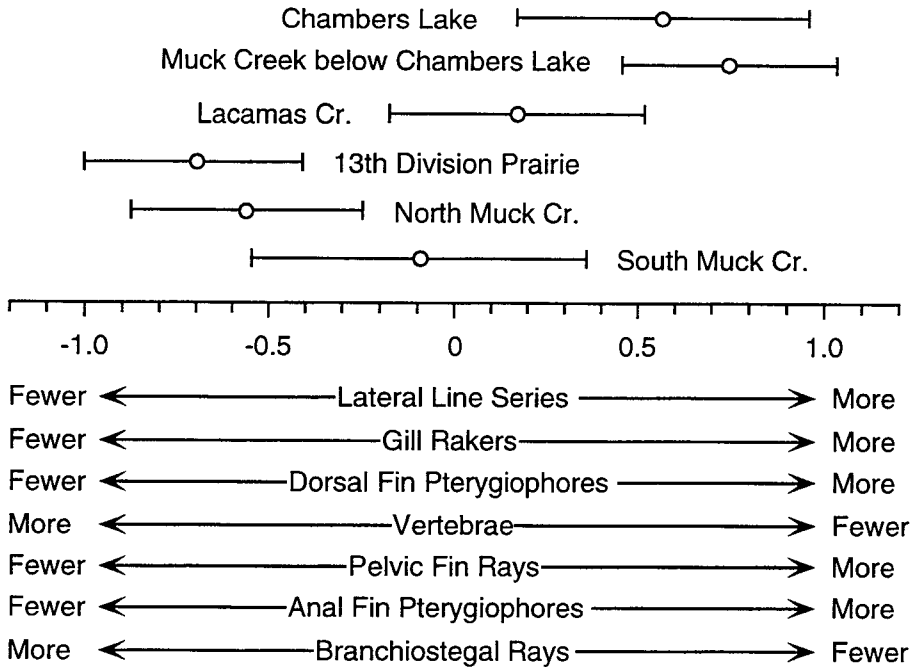


Figure 5. Means and 95% confidence intervals of gill raker (A) and branchiostegal ray (B) counts of coastal cutthroat trout in the Muck Creek Basin, Washington.

A) Discriminant Function 1 Scores



B) Discriminant Function 2 Scores

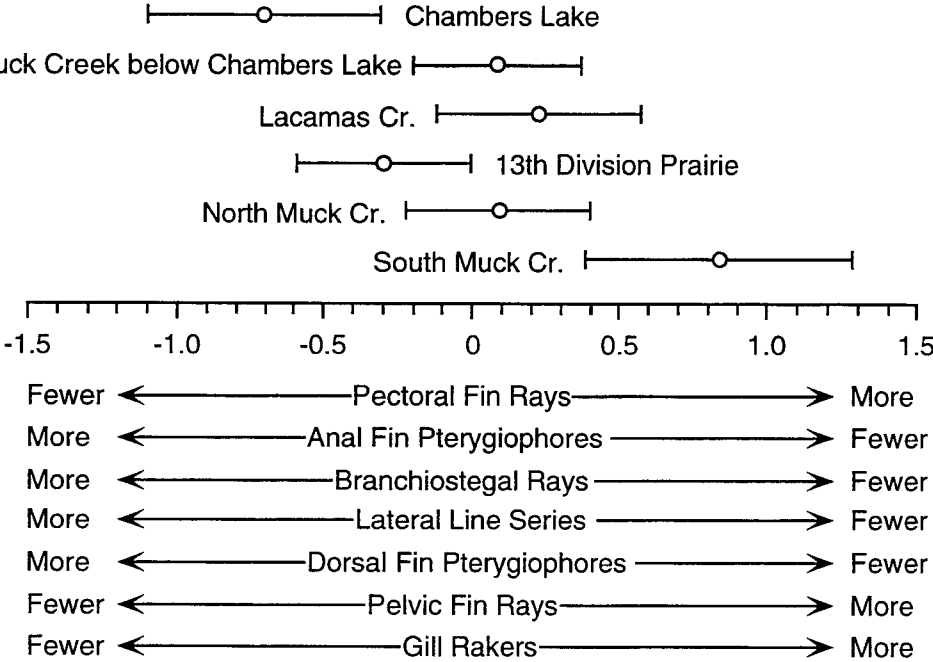


Figure 6. Means (centroids) and 95% confidence intervals on (A) the first, and (B), the second discriminant function axis for coastal cutthroat trout meristic characters, Muck Creek Basin, Washington.

Table 11. Significance (P-value) of the first discriminant function values above diagonal and second discriminant function values below diagonal, of coastal cutthroat trout, Muck Creek basin, Washington (*= $P > 0.05$, n.s.= not significant).

Sample ¹	1	2	3	4	5	6
1	----	*	n.s.	*	*	*
2	n.s.	----	n.s.	*	*	n.s.
3	*	*	----	*	*	*
4	n.s.	*	n.s.	----	n.s.	*
5	n.s.	n.s.	*	n.s.	----	n.s.
6	*	*	*	*	*	----

¹ 1=Muck Creek below Chambers Lake, 2=Lacamas Creek, 3=Chambers Lake, 4=13th Division Prairie, 5=North Muck Creek, 6=South Muck Creek

The percent classification by sample site demonstrates the separation of upper and lower sample sites (Table 12). Lower basin sites were most frequently misclassified as other lower sample sites and upper sites were most frequently misclassified as upper sites with the exception of Lacamas Creek. Lacamas Creek fish were equally misclassified as South Muck Creek fish.

Table 12. Discriminant function classification results for meristic characters of coastal cutthroat trout from various locations in the Muck Creek basin, Washington.

Actual Group ¹	Predicted Group ¹ (count, percentage)						Total
	1	2	3	4	5	6	
1	24 51.06	4 8.51	6 12.77	3 6.38	2 4.26	8 17.02	47 100.00
2	7 21.88	10 31.25	3 9.38	3 9.38	4 12.50	5 15.63	32 100.00
3	3 12.00	4 16.00	13 52.00	3 12.00	1 4.00	1 4.00	25 100.00
4	3 6.82	1 2.27	8 18.18	15 34.09	9 20.45	8 18.18	44 100.00
5	1 2.56	5 12.82	3 7.69	10 25.64	13 33.33	7 17.95	39 100.00
6	1 5.26	2 10.53	1 5.26	2 10.53	3 15.79	10 52.63	19 100.00

¹ 1=Muck Creek below Chambers Lake, 2=Lacamas Creek, 3=Chambers Lake, 4=13th Division Prairie, 5=North Muck Creek, 6=South Muck Creek.

DISCUSSION

Structure of Populations

Patterns of allelic and meristic variation indicate significant differentiation among coastal cutthroat trout populations in the Muck Creek basin. There is a weak differentiation between sites above and below Chambers Lake and a stronger separation between the Chambers Lake site and all other sites. Such a structuring is suggested by both genetic and meristic characters, and there is agreement between the two methods. For example, the second discriminant function (Figure 6B) illustrates a pattern similar to that of the dendrogram of genetic differences (Figure 2A). In the absence of such a structuring, all sample sites would be expected to resemble each other with no significant differences in allele frequencies or meristic mean values. These findings also suggest a separation between resident and anadromous populations.

Within basins, a structuring of populations can occur as a result of reproductive isolation. Reproductive isolation can result from barriers to migration such as waterfalls or behavioral mechanisms related to the timing and location of spawning. Reproductive isolation resulting from geologic barriers to migration such as waterfalls has been shown to lead to genetic differentiation in rainbow trout (Northcote et al. 1970; Currens et al. 1990). However, such barriers do not exist in the Muck Creek basin. Reproductive isolation among populations of coastal cutthroat trout in the Muck Creek basin is probably maintained by spatial and temporal mechanisms. The low flow barrier above Chambers Lake probably serves to isolate populations above and below Chambers Lake. During the two years covered by this study, the South Muck Creek and North Muck Creek populations appeared to spawn later than Chambers Lake and Muck Creek below Chambers Lake populations. Newly constructed redds were observed in the area immediately upstream of Chambers Lake in February 1993 and in South Muck Creek a redd was observed in April 1993.

The dendrogram containing the Bockette Creek data as an outgroup (Figure 2B) depicts a slightly different structure among sites within the Muck Creek basin because it

includes only 37 loci. The dendrogram containing only the Muck Creek basin sites (Figure 2A) was based on the data of 52 loci. In either case, the separation of the Chambers Lake sample from the other Muck creek basin samples is significant. In Figure 2B, the genetic distance between Chambers Lake and the other sites is close to one fifth the distance between Bockette Creek, a basin located approximately 400 km by water, and the Muck Creek basin sites. This serves to underscore the differences observed in the Chambers Lake sample.

Genetic Variation and Expression of Anadromy

The relation between anadromous and resident populations is suggested by the relation between Chambers Lake and the other sample sites. The Chambers Lake sample represents an anadromous population, the Muck below Chambers appears to be a mixed population, and the other sites appear to contain only resident individuals based on size and appearance. It is likely, however, that the Lacamas Creek sample could include anadromous individuals. Coastal cutthroat trout are known to migrate into non-natal streams to feed and overwinter (Johnston 1981; Jones 1977) and, therefore, it is possible that the Chambers Lake sample consists of strays from other systems. This does not seem likely, as the Chambers Lake sample did not deviate from the assumptions of Hardy-Weinberg equilibrium and, therefore, appears to be a separate local population. Wholly anadromous populations, such as the Chambers Lake population, appear to be highly differentiated from mixed and wholly resident populations.

This study suggests that anadromous and resident coastal cutthroat trout do not exist as wholly separate populations, but may also exist in mixed populations. The Chambers Lake population appeared to consist of a single anadromous population which is significantly different from the other sample sites. The Muck Creek below Chambers Lake population also contained anadromous individuals but did not differ significantly in allele frequencies from the other sample sites with the exception of Chambers Lake. The allele frequencies of the anadromous individuals in the Muck Creek below Chambers Lake sample were not similar to those from Chambers Lake. Thorpe (1987) suggested that the

timing of life history events, such as smolting and migration, of salmonids is genetically controlled under environmental instructions. Given such a control, residency and anadromy are determined by growth rate which is mediated by food availability. The mixture of anadromous and resident fish in the Muck Creek below Chambers Lake reach may be a result of differential growth rates leading to an emigration of some juveniles from the system as smolts. Faster growing individuals remain in the reach as a resident component of the population. In the reaches above Chambers Lake the genetic control of residency may be more fully developed because emigration from these reaches is not countered with a return of mature anadromous adults due to the timing of low flow and dry conditions. This is supported by Northcote's premise that genetic control of anadromy is strongest in habitats where emigration could be disadvantageous (Northcote 1992).

Genetic Variation Within Populations

Levels of heterozygosity were low in this study when compared to coastal cutthroat trout populations in northern Puget Sound (Campton 1981). Campton reported levels from 0.0378 to 0.1236, with most values falling between 0.07 and 0.11. Levels of heterozygosity were, however, similar to those observed in southern Oregon coastal streams (Currens et al. 1992). Low levels of heterozygosity, fewer rare alleles, and more loci fixed for a single allele should occur in small, isolated populations which are subject to inbreeding and random genetic drift. These conditions are likely to be typical of coastal cutthroat trout populations in small streams in southern Puget Sound.

Hutchings and Ferguson (1992) tested the relationship between allozyme heterozygosity and several life history traits including age and size at maturity, egg size, fecundity, and proportional allocation of body tissue to gonads within five populations of brook trout in Newfoundland, Canada. Life history traits examined were determined to be independent of allozyme heterozygosity. Hutchings and Ferguson (1992) concluded that environmental variation was likely to mask the effects that heterozygosity might have on the phenotype and developmental stability of life history traits. Further, they suggest that heterozygosity is likely to be associated with intermediate traits rather than extremes. In the expression of anadromy and residency, this relation suggests that if these life history tactics

are genetic, increased heterozygosity would be related to mixtures of anadromous and resident individuals within a single population. To test this relation, one would need to estimate the proportion of anadromous individuals within a population. This was attempted based on otolith micro-microchemistry but was not possible in this study (Appendix A).

Given the possible small effective population size of the Chambers Lake population, one would expect the effects of random genetic drift to be greater leading to significantly different allele frequencies when compared to the other sample sites which are potentially characterized by larger population sizes. This serves to underscore the importance of maintaining all populations of cutthroat trout in a basin as well as maintaining the possible links between resident populations.

Meristic Discrimination of Populations

Meristic characters may be affected by both genetic and environmental factors. In rearing experiments with steelhead, Winter et al. (1980) determined that the number of vertebrae and lateral line series scales were genetically influenced. Environmental factors affecting meristic characters include temperature, light, and dissolved oxygen (Winter et al. 1980). It is important to consider such factors when examining population structure as inferred from meristic analysis. MacGregor and MacCrimmon (1977) reported lower mean dorsal fin, anal fin, and branchiostegal ray counts and higher mean pectoral fin ray counts at higher incubation temperatures. No significant effect of water temperature was observed in the number of vertebrae. Given such a relationship, the Chambers Lake and Muck below Chambers samples would be expected to have lower counts than the sites located higher within the river continuum (Vannote et al. 1980). It would be reasonable to assume that the Lacamas Creek sample should be similar to the North and South Muck Creek samples as the three sites lie within the same portion of the stream continuum. Instead, the Lacamas Creek sample groups with the other lower sample sites in several characters. It appears, then, that the patterns of meristic variation are the result of genetic differences among the sample sites and represent a legitimate technique for delineating populations in the Muck Creek basin.

Management Implications

Management of the Muck Creek basin should consider the population structure of coastal cutthroat trout. The most significant issues currently affecting fish populations relate to habitat and movement of fish past the Chambers Lake dam. Consideration of the population structure should effect how the system is managed. For example, habitat improvements to South and North Muck Creeks should not be expected to affect anadromous populations.

Harvest is not a significant factor as the entire system is managed as a catch-and-release fishery. Most, if not all, fishing pressure occurs at Chambers Lake and Johnson Marsh due to ease of access and larger size of fish. As a result, any mortality resulting from this fishery is focussed on these populations. These populations may also be impacted by large populations of warmwater fishes, such as bass. Efforts to increase populations of anadromous coastal cutthroat in the basin will need to address these issues.

Anadromous populations (Chambers Lake and Muck Creek below Chambers Lake) are also significantly impacted by the dam at Chamber Lake. During the two years of this study, the reach just below the dam at Chambers Lake was dry during critical times for anadromous fish. In 1992, as this stretch was desiccating, juvenile steelhead and coastal cutthroat trout were stranded and predation by wading birds was high. Eventually, the entire stretch between the dam and the town of Roy was dry and all stranded fish were dead. The dam at Chambers Lake could be useful in maintaining flow through this reach during the times of migration for juvenile and adult steelhead and coastal cutthroat trout. Efforts to improve habitat for anadromous coastal cutthroat trout should focus on Chambers Lake, the area just upstream of Chambers Lake, and on Muck Creek below Chambers Lake.

This study reinforces the view that the practice of managing whole basins as single genetic units is not sufficient (Ståhl 1987). Within basins, there may exist genetically isolated populations of coastal cutthroat trout requiring specific management practices. In a metapopulation context, protection of each section within the watershed, as well as maintenance of connections between the sections, will provide sources of colonizing individuals in the event of a local extinction of coastal cutthroat trout within one section of the basin.

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APPENDIX

Use of Otolith Microchemistry to Discriminate Life History Morph and Maternal Origin of
Anadromous and Resident Coastal Cutthroat Trout (*Oncorhynchus clarki clarki*) in
Muck Creek, Washington

INTRODUCTION

Pacific salmonids (*Oncorhynchus* spp.) exhibit a variety of life history tactics including anadromy and residency. Pink salmon (*O. gorbuscha*) and chum salmon (*O. keta*) exhibit only anadromous phenotypes. Chinook salmon (*O. tshawytscha*) and coho salmon (*O. kisutch*) exhibit obligate anadromy with some individuals (precocial males) exhibiting a resident-like life history. On the other hand, steelhead trout (*O. mykiss*), coastal cutthroat trout (*O. clarki clarki*), and sockeye salmon (*O. nerka*) exhibit dual life history forms which are often sympatric in the same stream. The relationship between these sympatric life history morphs is poorly understood, yet has significant implications to management.

Resident and migratory forms may represent "ecophenotypes" within a single gene pool or may represent genetically distinct populations. Some genetic control of anadromy or residency has been identified by several investigators for a variety of species (e.g., Jonsson 1982; Foote et al. 1989; Skaala and Nævdal 1989). Other studies, however, indicate that both genetic and environmental factors are involved in the control of resident or migratory behavior (e.g., Nordeng 1983; Jonsson 1985).

Coastal cutthroat trout inhabit coastal streams from the Eel River in northern California to Prince William Sound in Alaska (Behnke 1992). Throughout this range, cutthroat trout exhibit anadromous, potomadromous, and resident life history patterns. Anadromous cutthroat trout migrate to saltwater and return to their natal stream to spawn. After the initial migration to saltwater, anadromous cutthroat trout often conduct annual migrations to saltwater, returning to freshwater each year to spawn or overwinter (Sumner

1953; Armstrong 1971; Giger 1972; Jones 1977; Johnston 1982). Non-anadromous populations exist as lacustrine and stream resident forms. Lacustrine forms migrate to lakes within the same basin to rear and return to their natal stream to spawn. Stream resident forms remain within their natal stream (usually small headwater streams).

Several investigators have speculated that headwater populations of resident cutthroat trout may contribute to the anadromous population (e.g., Jones 1977; Michael 1983). The extent of segregation between resident and anadromous forms of coastal cutthroat is unknown. Studies of segregation between life history morphs requires proper identification of each life history morph. A genetic study of coastal cutthroat trout in the Muck Creek basin, Washington was initiated in 1993 and a method of identification is required to distinguish the life history morphs in this system. Further, due to small population size, it is not possible to collect only adult fish. It is, therefore, necessary to assume the origin of juveniles collected for genetic analysis. A method of identifying maternal origin would allow for proper assignment of juveniles to anadromous or resident populations.

The chemical composition of otoliths and scales has been used to identify migratory and non-migratory stocks of several species. Bagenal et al. (1973) examined strontium (Sr)/calcium (Ca) ratios of brown trout (*Salmo trutta*) scales to distinguish resident and sea-run fish. Nelson et al. (1989) utilized carbon and oxygen isotopic composition to distinguish migratory and non-migratory stocks of New Zealand common smelt (*Retropinna retropinna*). Both of these techniques provide information on the life history of adult fish, but because the methods involve digesting the entire otolith or scale, it is not possible to determine elemental composition at a particular time. Given the concentric growth patterns of these structures and formation of distinguishable banding patterns associated with seasonal patterns, otoliths and scales provide a unique record of the life-history of an individual. Such banding patterns are commonly used to age fish and infer life-history events such as spawning and entry to saltwater.

Kalish (1990) and Reiman et al. (1994) used x-ray microprobe analysis to map transects of Sr/Ca ratios in otoliths of salmonids and, therefore, determine anadromy or residency. Examination of Sr/Ca ratios in the primordia were used to determine maternal contribution (anadromous or resident) based on the assumption that primordia composition would reflect the environment in which yolk precursors developed (in the ocean for anadromous morphs). Using these techniques, Reiman et al. (1994) were able to distinguish between anadromous sockeye salmon and kokanee in the Snake River, Idaho. This study was initiated to test the utility of such techniques to distinguish anadromous and resident life history morphs and maternal origin in coastal cutthroat trout in the Muck Creek Basin, Washington.

METHODS

Study Area

Muck Creek is a third order stream located in the southern Puget Sound region of western Washington (Figure 1). As a tributary to the Nisqually River, entering at river km 17, the Muck Creek watershed encompasses 238 km². The drainage has been moderately developed with rural residential homes, farms, and pastureland. Undeveloped portions consist of second growth coniferous, deciduous forest, and prairie (Williams et al. 1975). Most of the lower 11 km of Muck Creek lies within the Fort Lewis Military Reservation. The stream is low gradient and consists primarily of pool habitats, glide habitats, small lakes, and several marshy areas distributed throughout the drainage. Muck Creek experiences low flow conditions during the late summer and portions of the stream are dry for a month or more each year. Chambers Lake is a 40.5 ha lake created by a small dam just upstream from the town of Roy. The system is currently known to support cutthroat trout, steelhead, chum salmon, coho salmon, largemouth bass (*Micropterus salmoides*), sunfish (*Lepomis* spp.), yellow perch (*Perca flavescens*), and threespine stickleback (*Gasterosteus aculeatus*).

Otolith Analysis

Fish were collected from Chambers Lake with gill-nets and from South Muck Creek by electrofishing. Otoliths were removed in the lab and stored in 70% ethanol until preparation for microprobe analysis. The right otolith from each fish was mounted sulcus side down with Crystal Bond 509 (Aremco products Inc.) and ground in the sagittal plane to the nucleus with 600-grit and 1200-grit sandpaper. The mounting medium was then heated and the otolith turned sulcus side up. The otolith was then ground with 1200-grit and 2000-grit sandpaper to the level of the primordia and polished with a slurry of 0.05 μm

alumina paste. All otoliths were cleaned with deionized water, air-dried, and coated with a 200 Å carbon layer.

Elemental analysis was conducted with a Cameca SX-50 wavelength dispersive microprobe. A 15 kv, 50 nA, 7 µm diameter beam was used for all analyses. Strontiantite (SrCO₃-USNM R10065) and calcite (CaCO₃-USNM 136321) were used as standards for Sr and Ca, respectively. Each element was analyzed simultaneously and a counting time of 40s was used to maximize precision (Toole and Nielsen 1992). To measure reproducibility, a transect of 10 points was run along a single circulus. The SD of the 10 points indicates reproducibility of the microprobe given the assumption that the elemental composition of Ca and Sr is constant along a circulus. Counting statistics (1σ) and lower limits of determination (6σ) were calculated according to Potts (1987). Because Sr and Ca are reported as atomic ratios, the counting error is additive. Microprobe analyses were conducted on line transects bisecting a primordium and continuing to the edge of the otolith.

Otolith regions were classified as primordia, freshwater growth region, and saltwater growth region. Freshwater and saltwater growth regions were identified by growth rates inferred from banding patterns according to the standard methods of otolith analysis used in our lab and Fuss (1982). Each transect of Sr/Ca ratio was plotted and compared to expected transects for anadromous and resident fish as presented by Kalish (1990). Differences in Sr/Ca ratios were tested for significance using unpaired two-tailed *t*-tests comparing: Chambers Lake and South Muck Creek primordia, freshwater and saltwater growth regions in Chambers Lake, and freshwater growth regions in Chambers Lake and South Muck Creek.

RESULTS

Based on banding patterns, all Chambers Lake fish exhibited saltwater growth in addition to freshwater growth and all South Muck Creek exhibited only freshwater growth. The lengths and weights (Table 13) also indicated that the Chambers Lake and South Muck Creek samples represented anadromous and resident life history morphs, respectively.

Counting error associated with Ca was low (0.00093) as most counting error was attributable to Sr (0.096). Counting error of Sr/Ca ratios was 0.0001. Reproducibility of microprobe measurements was high and is reasonable when compared to the 1σ counting statistics (SD of 10 points on single circulus = 0.0001). Lower limits of determination for Ca and Sr were 0.010 and 0.0076, respectively.

Sr/Ca ratios in primordia, freshwater growth regions, and saltwater growth regions are presented in Table 13. Analysis of Sr/Ca ratios does not differentiate between the assumed anadromous and resident populations (Figure 7). Primordia of Chambers Lake and South Muck Creek fish did not significantly differ in Sr/Ca ratios (two-sided $p = 0.7682$; Figure 7). In otoliths of Chambers Lake fish, the ratio of assumed saltwater growth ($x = 0.00109$) was not significantly different compared to assumed freshwater growth regions ($x = 0.00099$; two-sided $p = 0.99$).

Table 13. Fork length (FL), weight (Wt), primordium Sr/Ca ratio, and mean (SD) Sr/Ca ratios in freshwater (FWG) and saltwater (SWG) growth regions in otoliths from coastal cutthroat trout, Muck Creek Basin, Washington.

<u>Fish</u>	<u>FL(mm)</u>	<u>Wt(g)</u>	<u>Primordium</u>	<u>FWG</u>	<u>SWG</u>
Chambers Lake					
931138	221	135	0.00096	0.00092 (0.00011)	0.00111 (0.00010)
931139	369	600	0.00108	0.00110 (0.00011)	0.00133 (0.00016)
931142	280	286	0.00122	0.00117 (0.00018)	0.00108 (0.00013)
931145	234	156	0.00134	0.00096 (0.00016)	0.00113 (0.00014)
931175	396	745	0.00097	0.00091 (0.00013)	0.00101 (0.00013)
931178	303	371	0.00104	0.00084 (0.00013)	0.00111 (0.00015)
942810	428	986	0.00156	0.00103 (0.00029)	0.00105 (0.00018)
942813	227	131	0.00249	0.00127 (0.00044)	0.00099 (0.00014)
South Muck Creek					
931727	187	75	0.00121	0.00127 (0.00012)	
931731	113	17	0.00133	0.00133 (0.00014)	
931733	116	17	0.00158	0.00131 (0.00015)	
931735	90	7	0.00141	0.00149 (0.00014)	
931739	87	7	0.00143	0.00139 (0.00014)	
931741	108	14	0.00119	0.00139 (0.00024)	
931743	85	6	0.00142	0.00134 (0.00013)	
931745	96	10	0.00154	0.00133 (0.00016)	

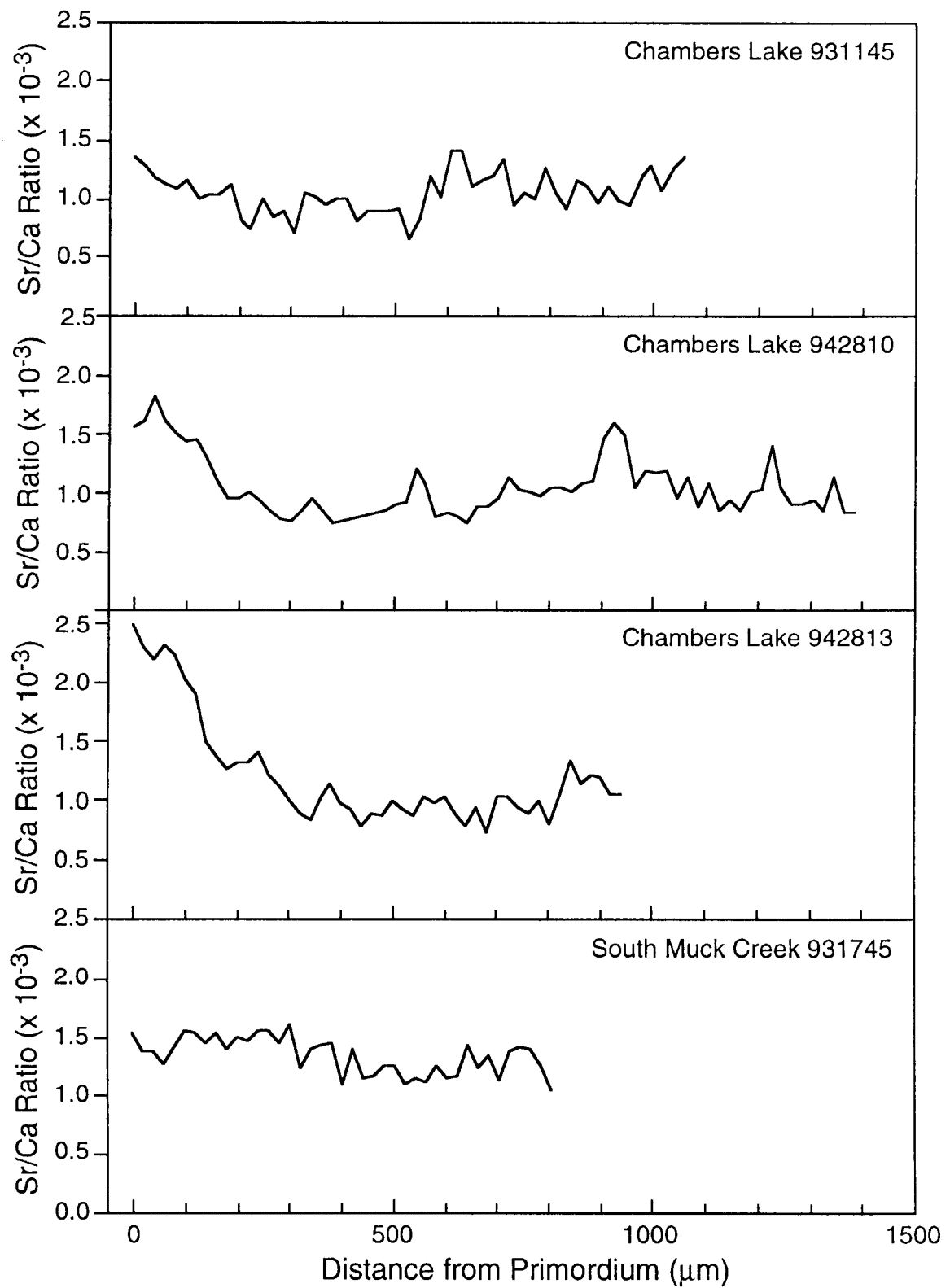


Figure 7. Life history transects of Sr/Ca ratios in coastal cutthroat trout otoliths from the Muck Creek Basin, Washington.

DISCUSSION

Analysis of otolith microchemistry is not an appropriate method of discriminating between resident and anadromous life history patterns in coastal cutthroat trout in the Muck Creek basin. The transects of Sr/Ca ratios obtained in this study (Figure 8) do not match those expected according to Kalish (1990). For example, fish 931145 appeared to show growth expected from an anadromous fish based on otolith microstructure, but the transect of Sr/Ca ratios is indistinguishable from that of resident fish 931745. In addition, the mean Sr/Ca ratio of freshwater growth in South Muck Creek was slightly higher than the mean Sr/Ca of assumed saltwater growth in the Chambers Lake fish (Figure 7). This indicates that the method is not appropriate in this situation. This may be due to low Sr content of South Puget Sound waters. Tagging studies indicate that coastal cutthroat trout do not migrate to the open ocean, but instead remain near river mouths and other estuarine areas (Johnston and Mercer 1976; Wydoski and Whitney 1979; Campton and Utter 1985). Anadromous fish from Muck Creek are, therefore, likely to remain near the mouth of the Nisqually River which lies at the southern-most extent of Puget Sound. This region is probably characterized by low salinity due to high freshwater input from local rivers and its distance from any saltwater inflow (the Strait of Juan De Fuca in the northern most region of Puget Sound). Chemical analysis of Sr and Ca in these waters would serve to confirm this assumption.

Sr/Ca ratios in the primordia of Chambers Lake and South Muck Creek were not significantly different and suggest that such analysis is incapable of determining maternal origin in this case (Figure 7). Fish 942813 was the single sample from Chambers Lake which showed a significant spike in Sr/Ca ratio in the primordium (Table 13; Figure 8). This fish could be the progeny of a straying female or may have strayed to the Muck Creek basin itself.

It is possible that the Chambers Lake fish are not anadromous, but rather potomadromous. In this case, they would be the progeny of resident fish that migrated to Chambers Lake. This suggests that the greater growth interpreted as saltwater growth is actually accelerated growth associated with migrating to the lake to rear. Water chemistry analysis of Sr and Ca will provide evidence to support or reject this possibility. If Sr levels in Puget Sound are significantly higher than in the Muck Creek basin, it would suggest that these fish did rear in Chambers Lake and are not anadromous. This is unlikely because Chambers Lake experiences lethal temperatures in the summer months and a fish kill was experienced in 1992.

This study suggests that analysis of Sr/Ca ratios in the otoliths of coastal cutthroat trout in the Muck Creek basin does not provide a tool to discriminate between individuals of resident or anadromous life histories or maternal origin. Although it does not work in this situation, it does not suggest that the method does not work in other situations. Rieman et al. (1994) were able to distinguish life history and maternal origin in Redfish Lake and other populations of sockeye salmon with the exception of one lake (Alturas Lake) because it exhibited high Sr. That study, in addition to this study, suggest that a pilot study such as this is required to test the applicability of otolith microchemistry methods.

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