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Troy J Guy for the degree of Masters of Science in Fisheries Science presented on August 12, 2004.

Title: Landscape-Scale Evaluation of Genetic Structure among Barrier-Isolated Populations of Coastal Cutthroat Trout, *Oncorhynchus clarki clarki*

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Abstract Approved:

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Although the effects of extrinsic barriers to dispersal have increasingly been shown to play a large role in the structuring of contemporary genetic diversity, describing the relationship between landscape structure, stochastic disturbance, and genetic diversity remains a major challenge. Here, environmental features for 27 barrier-isolated populations (2,232 individuals) of coastal cutthroat trout from western Oregon are compared with data from seven microsatellite loci to examine how watershed-scale environmental factors shape genetic diversity. Isolated headwater populations of coastal cutthroat trout are strongly differentiated (mean F_{st} = 0.33), but intrapopulation microsatellite genetic diversity (mean number of alleles per locus = 5, mean He = 0.60) was only moderate. Differences in genetic diversity of fish from the Coast Range (mean alleles = 47) and Cascade Mountains (mean alleles = 30) (P = 0.02)

coincided with differences in regional landscape feature. Furthermore, scatter evident from isolation by distance plots within ecoregions indicated that population structure was primarily mediated by gene flow in the Coast Range, but in the Cascade Mountains, genetic drift the dominant factor influencing genetic patterns. Thus through comparisons between landscape structure and genetic diversity we demonstrate an example where physical landscape features play a substantial role in the structuring of genetic diversity.

Landscape-Scale Evaluation of Genetic Structure among Barrier-Isolated Populations of Coastal Cutthroat Trout, *Oncorhynchus clarki clarki*

by

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Troy J. Guy, Author

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CHAPTER 1: INTRODUCTION

Understanding the processes that control genetic variation in space and time is fundamental for conserving biological diversity and understanding the processes of species evolution (Waples 1991; Moritz 1994; Bernatchez 1995). Genetic analyses are commonly used to determine geographic structuring among populations that are spatially and temporally subdivided in a hierarchical manner by limited dispersal among local breeding populations (Hanski 1998). These local populations are subject to extinction through stochastic environmental dynamics and demographic effects such as bottlenecks, founder effects, and drift (Lacy 1987).

In salmonid fishes, genetic structuring has been shaped by large-scale historical events such as range expansions or contractions following glacial retreat and pluvial periods (Taylor et al. 2001; Nielsen and Sage 2002; Castric and Bernatchez 2003). Recent research suggests, however, that the contribution of contemporary landscape features to the structure of genetic diversity supersedes or moderates the historical genetic signature, at least on small spatial scales (Angers et al. 1999; Costello et al. 2003). For example, landscape features such as drainage network complexity and among-habitat elevation differences can limit dispersal among populations (Angers et al. 1999; Hebert et al. 2000; Castric et al. 2001). Increasingly, studies have shown patterns of genetic diversity to be strongly influenced by populations above migration barriers (Hebert et al. 2000; Costello et al. 2003; Taylor et al. 2003). Similarly, natural cascades and waterfalls can influence patterns of genetic variability by acting as filters or barriers to dispersal from adjacent

downstream populations (Currens et al. 1990; Griswold et al. 1997; Carlsson et al. 1999; Carlsson and Nilsson 2001).

Genetic diversity is generally assumed to be lower in populations isolated from gene flow because of demographic effects that act on populations of small effective sizes (Ne). However, the relative effects of ecological processes and habitat features (e.g., disturbance regime, habitat size, topologic complexity, and within-stream connectivity) on genetic diversity have not been thoroughly evaluated (Angers et al. 1999; Hebert et al. 2000; Castric et al. 2001). Headwater stream fishes existing above waterfalls provide a unique opportunity to examine the linkages between habitat features and population genetic structuring because their genetic composition is not confounded by serial dispersal from downstream populations, at least over shorter time scales.

Coastal cutthroat trout (*Oncorhynchus clarki clarki*), a salmonid subspecies commonly found in headwater streams of the Pacific Northwest, are an excellent model for examining the influence of landscape on genetic structure. Individual populations have adapted to climatic, hydrologic, and geomorphic conditions that vary across the historic range of the subspecies from Humboldt Bay, California to Prince William Sound, Alaska (Fig. 1). This region lies on an active tectonic margin characterized by large uplifting mountain ranges that are actively eroded by substantial winter precipitation (160-230 cm) and complex river systems with numerous small tributaries. Glacial ice covered the majority of the northern region as recently as 10,000 ybp, but coastal cutthroat trout may have persisted south of the Cordilleran ice

Figure 1. Sampling locations, geographic range, and relationship to the southern extent of Cordilleran ice sheet for coastal cutthroat trout.

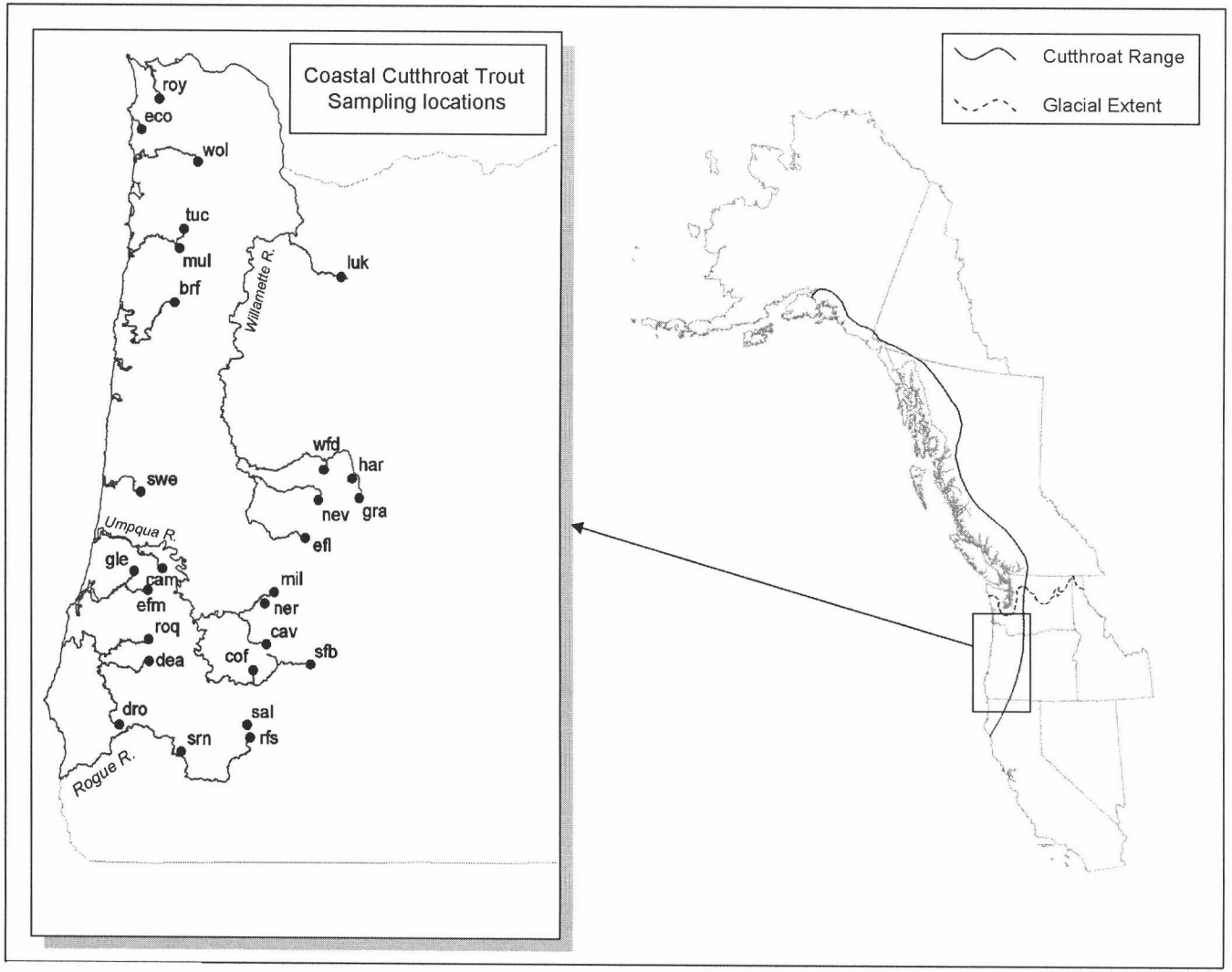
Coastal Cutthroat Trout
Sampling locations
roy
eco
wol
tuc
mul
brf
luk
Willamette R.
wfd
har
nev
gra
swe
Umpqua R.
efl
gle
cam
mil
efm
ner
roq
cav
dea
cof
sfb
dro
sal
srn
rfs
Rogue R.
Cutthroat Range
Glacial Extent

Figure 1

sheet in the Cascadia refugia for up to a million years (Fig. 1; McPhail and Lindsey 1986, Behnke 1992).

Coastal cutthroat trout exhibit a diverse array of life-history types including anadromous, amphidromous, potamodromous, and non-migratory forms (Trotter 1989). In the last few decades, range-wide declines in abundance and distribution have raised concerns about the long-term persistence of the subspecies, especially the anadromous form (Nehlsen et al. 1991; Trotter et al. 1993), and petitions were submitted to list coastal cutthroat trout under the Endangered Species Act (ESA). The relatively abundant potamodromous and non-migratory forms have received little attention despite the fact that they exist in complex assemblages of small headwater-populations, potentially isolated from downstream gene flow. These populations are especially vulnerable to large-scale landscape disturbances acting disproportionately on dendritic headwater stream networks (Fagan 2002).

Ecologists have long recognized the importance of disturbance regimes for creating and maintaining a mosaic of habitats that form the physical template for evolution (Southwood 1977; White and Pickett 1985). In the Pacific Northwest, mass wasting events, such as landslides and debris flows, are important processes for stream dwelling organisms, especially headwater fishes (Reeves et al. 1995). Landslides and debris flows are responsible for sediment and wood input to stream channels that provide complex habitat for stream fishes (Swanson et al. 1987; Benda and Dunne 1997; May and Gresswell 2004); however, these stochastic events can potentially extirpate, or severely reduce, local populations (Lamberti et al. 1991; Smith and Atkinson 1999; Roghair et al. 2002).

The interaction of disturbance and habitat formation is especially important for coastal cutthroat trout because strong population structuring is often apparent at all levels of population organization, even within individual streams and tributaries (Campton and Utter 1987; Wenburg and Bentzen 2001; Wofford et al. In Press). Coastal cutthroat trout are common in small streams and are frequently the only fish species encountered in headwater surveys (McPhail 1967; Wydoski and Whitney 1979; Reeves et al. 1998). Individuals often occur above waterfalls in relatively small isolated populations that have direct contact with mass wasting events (May and Gresswell 2003). Population demographics may rebound quickly following disturbance (Lamberti et al. 1991; Smith and Atkinson 1999; Gresswell 1999; Roghair et al. 2002), but genetic diversity will remain low if connectivity between neighboring populations is poor (Dunham et al. 1999).

Successful management for the persistence of coastal cutthroat trout requires a more thorough understanding of how contemporary environmental factors relate to genetic variation. Specifically, do differences in habitat structure and connectivity above barriers to migration translate to higher levels of genetic diversity? For example, do watersheds with greater connectivity and topologic network complexity retain more genetic diversity in spite of stochastic events such as mass wasting? To this end, we analyzed genetic variation in 27 isolated populations of coastal cutthroat trout across a diverse array of habitats in western Oregon. Study objectives were (1) to determine the extent of differentiation and hierarchical genetic structure among isolated coastal cutthroat trout populations in headwater streams and (2) assess how

watershed-scale environmental factors correlate with the structuring of genetic diversity.

CHAPTER 2: MATERIALS AND METHODS

Sample collection

In order to develop a sampling frame for known populations of headwater coastal cutthroat trout, we identified 269 headwater watersheds (approximately third order, 500 –1500 hectares) where populations of coastal cutthroat trout were the only salmonid above natural migration barriers, and there was no record of hatchery stocking (Gresswell et al. In Press). Because physiographic province and geology were expected to influence habitat/genetic relationships across western Oregon, six sampling strata were created by integrating the watersheds with Geographic Information System (GIS) coverages of ecoregions (Coast Range, Cascade Mountain, and Klamath Mountain ecoregions; Oregon Natural Heritage Program, 1:250,000 ecoregion coverage) and high and low erosion potential (reclassified USGS, 1:500,000 geology coverage). A random sample of 25 watersheds was selected for study in proportion to the number of watersheds in each stratum. Rainbow trout, *Oncorhynchus mykiss*, were visually identified in one watershed (Straight Creek) during sample collection, and this population was excluded from further analysis. Three additional populations (EF Laying Creek, Sweet Creek, and Camp Creek) were selected opportunistically to yield a total of 27 sample watersheds (Fig. 1; Table 1).

Because of logistical constraints of field sampling, and to avoid potentially comparing watersheds with large differences in effective population sizes (Ne), watershed size was restricted to 1,000 hectares. Camp Creek ($\approx$ 2,000 hectares), the focus of an intensive within-watershed genetic study (Wofford et al. In Press), is the

TABLE 1. Sampling date, location, and group classifications for coastal cutthroat trout for populations included in this study.

Creek Name	Code	n	Year	Northing	Easting	Geology	Ecoregion	ESU	Watershed	Strategy
Cavitt Creek	cav	94	2000	4776923.50	509350.34	hard	Cascade	Oregon Coast	Umpqua R.	Component
Coffee Creek	cof	95	2000	4763899.50	500567.38	soft	Cascade	Oregon Coast	Umpqua R.	Component
Miller Creek	mil	65	2000	4801471.00	507303.81	hard	Cascade	Oregon Coast	Umpqua R.	Complete
NF,EF Rock Creek	ner	19	2000	4808921.00	513817.53	hard	Cascade	Oregon Coast	Umpqua R.	Complete
SF Buckeye Creek	sfb	89	2001	4764867.50	535899.38	hard	Cascade	Oregon Coast	Umpqua R.	Component
EF Laying Creek	efl	96	2000	4837587.50	533377.75	hard	Cascade	Upper Willamette	Willamette R.	Component
Grasshopper Creek	gra	91	1999	4862816.00	566035.00	hard	Cascade	Upper Willamette	Willamette R.	Component
Hardy Creek	har	89	2000	4871860.50	561125.38	hard	Cascade	Upper Willamette	Willamette R.	Complete
Lukens Creek	luk	95	2001	4988354.00	559505.69	hard	Cascade	Upper Willamette	Willamette R.	Component
Nevergo Creek	nev	15	2000	4861509.00	540977.63	hard	Cascade	Upper Willamette	Willamette R.	Component
WF Deer Creek	wfd	74	2000	4878597.50	543431.19	soft	Cascade	Upper Willamette	Willamette R.	Complete
Bridge Forty Creek	brf	93	1999	4975023.00	452950.38	hard	Coast	Oregon Coast	Central Coast	Component
Sweet Creek	swe	88	2001	4865603.50	432341.28	soft	Coast	Oregon Coast	Central Coast	Component
EF Millicoma Creek	efm	94	2000	4807899.00	436663.63	soft	Coast	Oregon Coast	Coos R.	Component
Glenn Creek	gle	96	2000	4820370.50	428690.00	soft	Coast	Oregon Coast	Coos R.	Component
Dead Horse Creek	dea	96	2001	4779452.50	436894.16	soft	Coast	Oregon Coast	Coquille R.	Complete
Drowned Out Creek	dro	91	2000	4729015.50	418643.84	soft	Coast	Oregon Coast	Coquille R.	Complete
Rock Creek (Coquille)	roc	82	2001	4766737.50	437381.41	soft	Coast	Oregon Coast	Coquille R.	Component
NF Ecola Creek	eco	96	2001	5076097.00	431525.69	hard	Coast	Oregon Coast	North Coast	Component
Muletail Creek	mul	95	2000	5007318.00	453145.69	soft	Coast	Oregon Coast	North Coast	Complete
Tucca Creek	tuc	96	1999	5018858.00	458526.22	soft	Coast	Oregon Coast	North Coast	Component
Wolf Creek	wol	96	2000	5058416.50	467060.63	hard	Coast	Oregon Coast	North Coast	Complete
Camp Creek	cam	92	2000	4820829.50	445105.72	soft	Coast	Oregon Coast	Umpqua R.	Complete
Rock Creek (Youngs)	roy	94	2000	5093481.00	442359.16	soft	Coast	SW WA /Columbia R	North Coast	Component
RF Salt Creek	rfs	86	2001	4722844.00	499061.06	hard	Klamath	S. Oregon / N. Calif.	Rogue R.	Component
Salt Creek	sal	83	2001	4727443.00	499260.63	hard	Klamath	S. Oregon / N. Calif.	Rogue R.	Component
Little Stratton Creek	srn	32	2001	4713908.00	456924.09	soft	Klamath	S. Oregon / N. Calif.	Rogue R.	Component

only exception to this criterion. For the remaining 26 watersheds, the size was restricted in two ways. First, sampling was initiated immediately above the isolating barrier if the drainage was 500-1,000 hectares. This situation will be referred to as the "complete" watershed sampling strategy. Second, if the drainage area above the isolating barrier was >1,000 hectares, sampling was initiated at the first tributary junction (working progressively upstream) that drained a watershed meeting the size criterion. Similarly, if the watershed contained two or more subwatersheds between 500 and 1,000 hectares, one watershed was randomly selected for sampling. The watersheds in the second group were collectively assigned the term "component" watershed sampling strategy (Table 1). Results were summarized separately for complete and component watersheds, and subsequently for all watersheds combined.

Genetic samples were collected from coastal cutthroat trout from each of the 27 watersheds between 1999 and 2002. Fish were collected using single-pass electrofishing techniques starting in the lowest portion of each of the watersheds and continuing upstream (Bateman et al. In Review). To capture a large portion of the genetic diversity from each sampling location (Banks et al. 2000), fin tissue was taken from up to 96 fish per watershed. In some locations, the upper extent of fish distribution was reached before 96 samples were obtained. In these cases, a large percentage of the population was sampled (Bateman et al. In Review), and we assume these samples encompass the range of genetic variation in the population despite lower overall sample sizes. In order to reduce the chances of family sampling (Hansen et al. 1997), tissue collections were restricted to fish >50 mm in length and were staggered spatially by sampling up to 10 fish in 10-mm size classes from each geomorphic

segment (Frissell et al. 1986; Montgomery and Buffington 1997) until 96 samples were obtained or the upper extent of fish distribution was reached. Captured fish were anesthetized with clove oil to reduce handling stress (Taylor and Roberts 1999). Each fish was weighed and measured, and a small portion of the caudal fin was removed and stored in a desiccant (anhydrous sulfide crystals) or a buffer solution (100mM trisHCl pH8, 100mM EDTA pH8, 10mM NaCl, 0.5% (w/v) SDS). Subsequently all fish were released to their source location.

Microsatellite analysis

Seven microsatellite loci in three multiplexed sets were chosen after screening for reliable PCR amplification, ease of scoring, and polymorphism (Table 2). Genomic DNA was extracted from fin tissue using Chelex®100 following the procedure of Banks et al. (2000). Each multiplex set was amplified with a MJ Research PTC-225 thermocycler under varying reagent concentrations and PCR conditions (Table 2; Appendix 1). The PCR products were analyzed on a 5% acrylamide gel using electrophoresis on a MJ Research Basestation DNA fragment analyzer (MJ Bioworks Inc.). Allele sizes were scored using the computer program Cartographer (MJ Geneworks, Inc.), verified visually, and hand corrected on screen as necessary.

Statistical analysis

Within-population parameters (sample size (n), number of alleles per locus (A), expected heterozygosity (He), observed heterozygosity (Ho), and allele

TABLE 2. Microsatellite loci with annealing temperatures (°C) in parenthesis, number of nucleotide repeats, primer concentration in PCR reaction, total number of alleles, allele size range, average expected heterozygosity, and original citation for loci in each multiplex used to assess variation in coastal cutthroat trout.

Multiplex (temp)*	Locus	Repeat	Primer (mM)	Allele	Size Range (bp)	Avg. He	Reference
Set A	One102	tetra	0.50	20	192-268	0.55	Olsen et. al 2000
(49)	One103	tetra	0.50	16	106-166	0.55	Olsen et. al 2000
	One108	tetra	0.50	31	138-266	0.59	Olsen et. al 2000
Set B	Omy77	di	0.50	30	97-169	0.62	Morris et. al. 1996
(56)	Ots4	di	0.08	11	107-129	0.47	Banks et. al. 1999
Set C	Ots209	tetra	0.08	11	139-195	0.54	Grieg et. al. 2003
(63)	Ots212	tetra	0.50	21	103-223	0.59	Grieg et. al. 2003

frequencies) were calculated using FSTAT version 2.9.3.2 (Goudet 1995). Pairwise Fst (theta) values (Weir and Cockerham 1984) were compared among locations and ecoregions using a permutation approach in FSTAT. To further evaluate results from a previously defined ecological basis, Fst values were calculated among evolutionary-significant-unit population groups (Waples 1991). In addition, pairwise Cavalli-Sforza Edwards chord distance (CSE) was calculated using the GENDIST program (PHYLIP (Phylogeny Inference Package), Felsenstein 2002). To test for differences in allelic frequencies between all population-pairs, Fisher's exact test was implemented with default parameters in GENEPOP (Raymond and Rousset 1995). Tests for deviations from Hardy-Weinberg equilibrium for each population by locus pair and for genotypic linkage disequilibrium for all combinations of locus pairs

within a population were performed using an exact test based on Markov chain iterations in GENEPOP with default parameters. Statistical significance was evaluated using Bonferroni adjusted P-values where appropriate (Rice 1989). Hierarchical partitioning of genetic diversity was assessed as the percent of variation explained by groupings into ecoregions, evolutionary significant units, and major hydrological watersheds (e.g., Willamette River and Umpqua River, Table 1), and between complete and component watersheds using an AMOVA procedure in Arlequin version 2.0 (Schneider et al. 1997).

Isolation–by-distance was assessed by examining correlation between genetic distance and geographic distance using Mantel tests in GENEPOP with default parameters. Both Fst and CSE pairwise genetic distance values were evaluated separately for all tests. Geographic distances among sampling locations were calculated as stream network distances using regional 1:100,000 hydrologic stream layers with ArcGIS software (version 8.2 Environmental Systems Research Institute). A second Mantel test was performed to test for migration-drift equilibrium when the initial isolation-by-distance analysis was positive. The second test was performed on the residuals from the initial fitted line and geographic stream distance (Hutchison and Templeton 1999; Costello et al. 2003; Taylor 2003). Isolation–by-distance tests were performed independently for complete, component, and all watersheds combined.

Because we were interested in potential effects of grouping populations by ecoregion or evolutionarily significant units, tests were repeated for populations partitioned into Coast Range and Cascade Mountain ecoregions and Oregon Coast and Upper Willamette River evolutionary significant units. Populations in the Klamath

Mountain ecoregion and in the Southwest Washington/ Columbia River and Southern Oregon/ California Coast evolutionary significant units were not included in these isolation-by-distance analyses because sample sizes were too low ($n < 3$). The degree of "scatter" (Hutchison and Templeton 1999) in the genetic distance metric in isolation-by-distance plots was compared among ecoregions and evolutionary significant unit groupings in order to ascertain the relative contribution of drift and gene flow in each grouping (Hutchison and Templeton 1999; Costello et al. 2003; Taylor 2003).

Environmental comparisons

Genetic diversity (i.e., total number of alleles for all loci) and physical environmental variables that we assumed to influence genetic structure (i.e., degree of isolation, watershed area, topological stream channel complexity, and within-watershed connectivity) were compared between the Coast Range and Cascade Mountain ecoregions using two sample t-tests (comparisons were conducted for complete, component, and all of the watersheds combined). We assumed that the degree of isolation was directly related to the vertical height of the waterfall barrier that isolated the watershed and that barrier height should reflect permeability to gene flow over time. Watershed area was assumed to influence potential differences in effective population size and was measured as drainage area above the barrier (km2). An index of topological stream channel complexity was developed using the ratio of summed tributary lengths to the longest length of stream (i.e., main stem) per watershed (m). Network complexity (i.e., more than one tributary of significant

length) was predicted to positively effect persistence following population losses in any single tributary resulting from a catastrophic event. In order to compare within-watershed connectivity, the total number of vertical falls (i.e., steps >1 m) were divided by the total number of habitat units per watershed (calculated from field surveys) for each watershed. Statistical analyses were conducted using Number Cruncher Statistical Systems software (NCSS; Hintze 2001).

CHAPTER 3: RESULTS

Within-population variation

Genotypes from 2,232 individual fish were successfully obtained from 27 sampling locations. Genetic variation was generally high despite the isolation of sampling sites above waterfalls. Number of alleles per locus for all populations ranged from 11 (*Ots4*) to 31 (*One108*), with a mean of 20 per locus. Mean expected heterozygosity was 0.60 [range: 0.47 (*Ots4*)-0.62 (*Omy77*)]. Tests for Hardy-Weinberg equilibrium at individual loci across populations (7 loci x 27 populations) revealed failures in 21 of 189 cases (11.1%) after Bonferroni corrections ($\alpha = 0.05/27 = 0.0019$). Failures were generally spread among all loci and populations, but a slight concentration was observed in *Omy77*. Failures were reduced to 9.3% with *Omy77* removed, but results from all subsequent analyses were not appreciably different. Therefore, *Omy77* was retained for all analyses. Tests for linkage disequilibrium yielded 10 departures for 567 comparisons (1.8%, $\alpha=0.05/27 = 0.0019$), and thus, no concentration of departures among locus pairs or within populations was detected.

Among-population variation and genetic structure

Genetic differentiation among populations was strong. Allelic frequency distributions from 351 pairwise population comparisons suggested significant population independence ($\alpha = 0.05/27 = 0.0019$), and the majority of comparisons by locus were also statistically significant ($\alpha = 0.05/7 = 0.007$; Table 3). Similarly, population genetic structure was strong. The mean Fst value was 0.33. The pairwise

TABLE 3. Genetic distance and allelic differentiation results for populations of coastal cutthroat trout included in this study. Values above the diagonal represent pair-wise Fst values; all estimates* were significant at the adjusted nominal level (5%) for multiple comparisons (alpha = 0.000132). Values below the diagonal represent the number of loci out of 7 (mil = 5) that revealed significant differentiation between populations after Bonferroni adjustments (alpha = 0.007).

	Population																										
	gle	roy	brf	dea	efl	wol	cav	luk	eco	tuc	mul	swe	roc	ner	mil	cam	har	gra	efm	sal	cof	dro	rfs	sfb	wfd	nev	stn
gle	~	0.11	0.10	0.21	0.13	0.33	0.13	0.19	0.05	0.09	0.18	0.08	0.13	0.18	0.13	0.15	0.47	0.27	0.18	0.27	0.19	0.12	0.27	0.55	0.55	0.20	0.20
roy	7	~	0.14	0.25	0.14	0.36	0.14	0.18	0.11	0.14	0.19	0.14	0.16	0.20	0.20	0.22	0.46	0.22	0.26	0.35	0.22	0.15	0.34	0.55	0.53	0.23	0.20
brf	7	7	~	0.28	0.13	0.36	0.13	0.18	0.09	0.10	0.17	0.10	0.10	0.23	0.18	0.21	0.53	0.25	0.18	0.33	0.21	0.12	0.33	0.53	0.52	0.20	0.20
dea	7	7	7	~	0.24	0.39	0.19	0.28	0.21	0.26	0.28	0.26	0.24	0.31	0.22	0.36	0.58	0.39	0.30	0.36	0.29	0.31	0.31	0.65	0.65	0.34	0.31
efl	7	7	7	7	~	0.29	0.15	0.17	0.12	0.14	0.22	0.13	0.13	0.25	0.20	0.21	0.46	0.26	0.21	0.34	0.20	0.17	0.33	0.54	0.50	0.15	0.16
wol	7	7	7	6	7	~	0.38	0.36	0.33	0.35	0.40	0.36	0.33	0.48	0.42	0.45	0.73	0.48	0.41	0.54	0.45	0.39	0.53	0.76	0.75	0.42	0.46
cav	7	7	7	7	7	7	~	0.20	0.12	0.15	0.18	0.13	0.13	0.23	0.16	0.25	0.47	0.27	0.26	0.30	0.19	0.16	0.28	0.56	0.55	0.25	0.16
luk	7	7	7	7	7	7	7	~	0.17	0.18	0.20	0.18	0.19	0.25	0.25	0.31	0.53	0.18	0.27	0.37	0.27	0.22	0.34	0.55	0.54	0.19	0.26
eco	7	7	7	7	7	7	7	7	~	0.05	0.15	0.04	0.10	0.19	0.09	0.15	0.45	0.23	0.19	0.27	0.18	0.08	0.28	0.52	0.52	0.21	0.19
tuc	7	7	7	7	7	7	7	7	3	~	0.18	0.09	0.15	0.23	0.19	0.22	0.47	0.22	0.22	0.30	0.22	0.12	0.31	0.54	0.53	0.19	0.21
mul	7	7	7	7	7	7	7	7	7	7	~	0.16	0.19	0.24	0.15	0.31	0.55	0.26	0.31	0.38	0.27	0.23	0.37	0.59	0.53	0.29	0.30
swe	7	7	7	7	7	7	7	7	7	7	7	~	0.11	0.21	0.13	0.14	0.51	0.24	0.21	0.33	0.16	0.11	0.33	0.51	0.53	0.25	0.23
roc	7	7	5	7	7	7	7	7	7	7	7	7	~	0.23	0.17	0.22	0.57	0.27	0.19	0.37	0.18	0.13	0.34	0.49	0.53	0.26	0.21
ner	5	6	7	7	7	7	7	7	5	5	7	7	7	~	0.21	0.33	0.77	0.37	0.33	0.43	0.27	0.25	0.42	0.84	0.83	0.37	0.37
mil	5	5	4	5	5	5	5	5	5	5	5	5	5	4	~	0.27	0.67	0.32	0.26	0.35	0.13	0.22	0.33	0.69	0.68	0.34	0.26
cam	7	7	7	7	7	7	7	7	7	7	7	7	7	6	5	~	0.61	0.41	0.30	0.47	0.28	0.17	0.47	0.67	0.68	0.38	0.36
har	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	~	0.58	0.64	0.62	0.59	0.52	0.64	0.94	0.93	0.76	0.65
gra	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7	~	0.38	0.44	0.36	0.29	0.45	0.63	0.59	0.36	0.36
efm	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7	7	~	0.37	0.30	0.25	0.39	0.59	0.65	0.34	0.31
sal	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7	7	7	~	0.34	0.37	0.09	0.76	0.74	0.48	0.34
cof	6	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7	7	7	7	~	0.24	0.29	0.61	0.63	0.39	0.24
dro	7	7	7	7	7	7	7	7	6	7	7	5	7	5	5	7	7	7	7	7	7	~	0.37	0.57	0.56	0.27	0.25
rfs	7	7	7	7	7	7	7	7	7	7	7	7	7	6	5	7	7	7	7	2	7	7	~	0.76	0.75	0.48	0.34
sfb	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7	7	7	7	7	7	7	~	0.98	0.87	0.76
wfd	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	6	7	7	7	7	7	7	7	~	0.88	0.76
nev	6	7	7	7	6	7	7	7	6	6	7	7	7	6	4	7	7	7	7	7	7	6	7	7	7	~	0.33
stn	7	7	7	7	7	7	7	7	7	7	7	7	7	6	5	7	7	7	7	7	7	7	7	7	7	6	~

*Miller Creek (mil) failed to amplify at Omy77 and Ots4, but Fst values were significant at 5 loci.

Fst values ranged from 0.04 to 0.98 ($P < 0.05$) (Table 3). Miller Creek was unique because *Omy77* and *Ots4* did not amplify in PCR reactions despite repeated attempts; apparently, this result was related to priming site mutations. Pairwise Fst comparisons among populations, including Miller Creek, were significant even after *Omy77* and *Ots4* were removed from the data.

Differences in hierarchical genetic variation among population grouped by ecoregions, evolutionary significant units, and major hydrological watersheds were statistically significant ($P < 0.05$), but in each comparison more genetic variation occurred within and among populations than among major groups (Table 4). Among ecoregion groups (Coast Range, Klamath Mountains, and Cascade Mountains), 5% of the variation was among groups, 27% among populations, and 68% within populations. The placement of populations into ecologically and genetically based evolutionary significant unit groups (Oregon Coast, Upper Willamette River, and Southern Oregon/ Northern California) produced very similar results. Among evolutionary significant unit groups, 7% of the variation was among groups, 27% among populations, and 67% within populations. Less of the variation was attributable to grouping by major watershed with 3% among groups, 28% among populations, and 69% within populations. Sampling strategy (complete or component) explained even less variation with <1% partitioned among groups, 32% among populations, and 68% within populations ($P = 0.15$).

TABLE 4. Hierarchical analysis of molecular variance (AMOVA) under varying regional groupings and between sampling strategies for coastal cutthroat trout included in this study. Values are expressed as the percentage of variation explained between groups, among groups and within populations. The stated P-value refers to the probability that the observed between group variation value is equaled or exceeed by chance from 1000 permutations. Probability for all observed values of variation among groups and within populations were 0.0001

Grouping	Between Group	Among Group	Within Population	Between Group P-value
Among Ecoregions	5	27	68	> 0.0001
Among ESUs	7	27	67	0.00
Among Watersheds	3	28	69	0.02
Complete vs. Component	1	30	69	0.12

Isolation-by-distance

An isolation-by-distance relationship was detected when all populations were combined ($r = 0.32$, $P < 0.01$; Fig. 2; Table 5). Similarly, a second Mantel test comparing residuals from the initial isolation-by-distance regression against stream distance was only positive when all watersheds were examined as a group ($r = 0.02$, $P < 0.018$). This result suggests that isolated coastal cutthroat trout populations in western Oregon (considered as a group) exhibit migration-drift equilibrium. Isolation-by-distance relationships were also detected when all component watersheds ($r = 0.29$, $P < 0.01$) and complete watersheds ($r = 0.55$, $P < 0.01$) were combined. A difference in the magnitude of isolation relationships in component and complete watersheds likely reflects the potential for smaller effective population sizes and greater genetic differentiation in the complete watersheds. When populations were

grouped by ecoregion, however, isolation-by-distance was only weakly detected for the Cascade

Figure 2. Isolation by distance plots across ecoregions and evolutionarily significant unit groupings for combined, component, and complete sampling strategies.

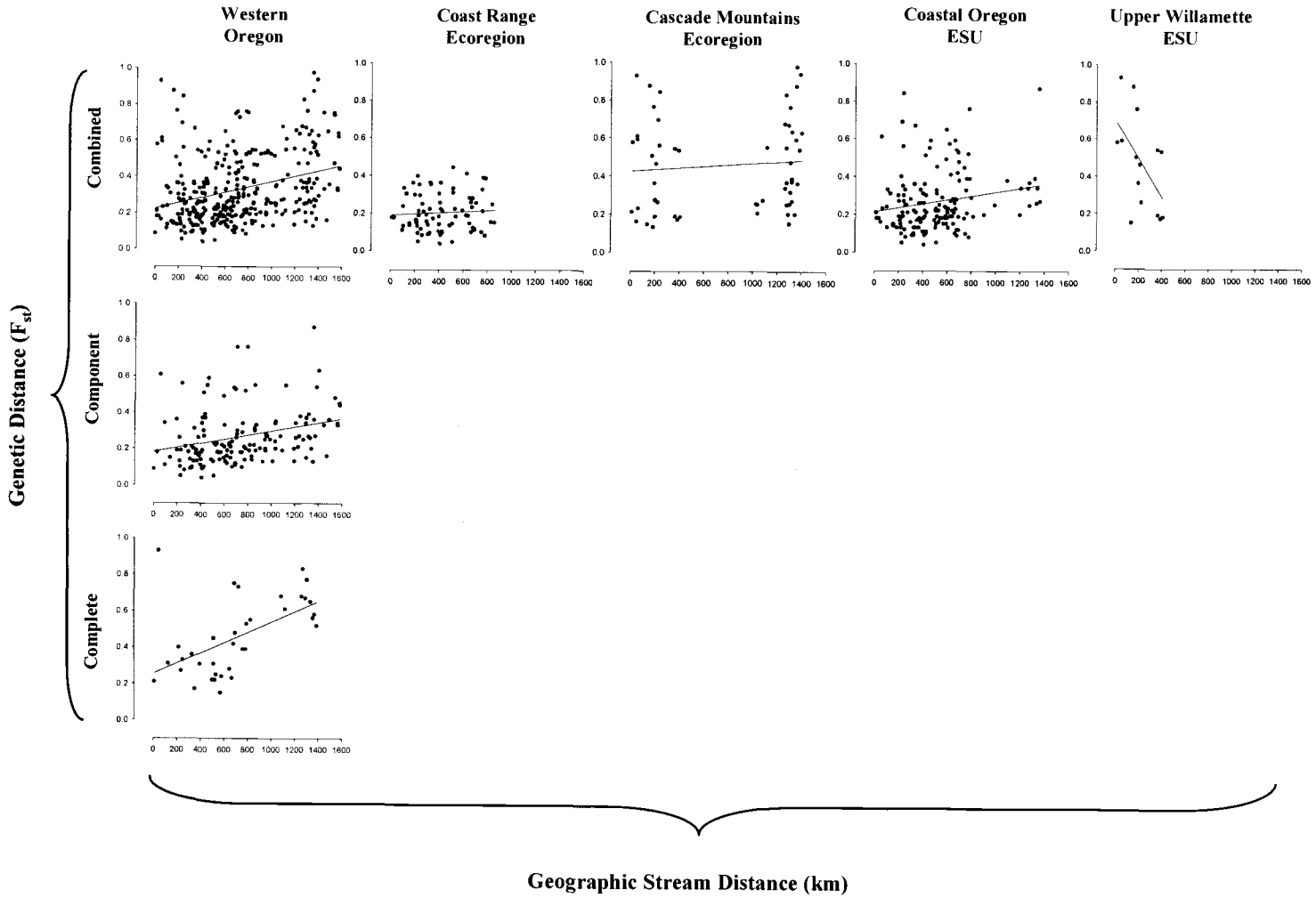
Western Oregon
Coast Range Ecoregion
Cascade Mountains Ecoregion
Coastal Oregon ESU
Upper Willamette ESU
Combined
Component
Complete
Genetic Distance (F_{st})
Geographic Stream Distance (km)

Figure 2

TABLE 5. Isolation-by-distance and average genetic distance for coastal cutthroat trout populations under varying sampling strategies that are grouped by ecoregion and evolutionarily significant unit. Correlation coefficients and P-values represent the relationship between genetic distance and geographic distance (r, Fst) or residuals and geographic distance plots (r, residuals) based on Mantel tests using 1,000 permutations. The results for all tests were consistent using either Fst or Cavalli-Sforza Edwards chord distance as the genetic distance metric (Fst presented).

Regional Grouping	Sampling Strategy	Populations (n)	r Fst	P Fst	r residuals	P residuals	Average Fst
All Populations Combined	All watersheds	27	0.32	< 0.01	0.02	0.02	0.33
	Component	18	0.29	0.00	0.01	0.63	0.27
	Complete	9	0.55	0.01	0.27	0.44	0.46
Coast Range Ecoregion	All watersheds	13	0.06	0.28	~	~	0.21
	Component	8	0.18	0.10	~	~	0.13
	Complete	5	0.13	0.10	~	~	0.33
Cascade Mountains Ecoregion	All watersheds	11	0.09	0.05	-0.06	0.82	0.46
	Component	7	0.24	0.01	0.12	0.31	0.35
	Complete	3	0.35	0.50	~	~	0.68
Oregon Coast Evolutionarily Significant Unit	All watersheds	18	0.20	0.08	~	~	0.27
	Component	10	0.26	0.07	~	~	0.24
	Complete	8	0.26	0.16	~	~	0.30
Upper Willamette Evolutionarily Significant Unit	All watersheds	6	-0.54	0.94	~	~	0.47
	Component	4	-0.38	0.54	~	~	0.22
	Complete	2	~	~	~	~	0.92

Mountain ecoregion ($r = 0.09$, $P < 0.05$), and a second Mantel test did not validate migration-drift equilibrium ($r = -0.06$, $P < 0.82$). None of the comparisons of isolation-by-distance for populations in the Coast Range ecoregion and the Oregon Coast and Upper Willamette River evolutionary significant units revealed a statistically significant relationship between geographic and genetic distance (Fig. 2; Table 5).

The degree of scatter and genetic differentiation (Fst) was strikingly different between ecoregions. For example, the scatter was consistently lower for the Coast

Range ecoregion than for the Cascade Mountain ecoregion (Fig. 2). In addition, mean Fst values in the Cascade Mountain ecoregion were consistently higher than in the Coast Range ecoregion (Table 5). These results suggest that each ecoregion is influenced differently by relative contribution from the forces of drift and gene flow (Hutchison and Templeton 1999). Genetic structure in the Coast Range ecoregion is apparently dominated by gene flow, and in the Cascade Mountains, drift appears to be the principal factor influencing genetic organization. Furthermore, scatter among populations in the Oregon Coast evolutionary significant unit was greater than displayed in the Coast Range ecoregion, and it appears that environmental factors may be more useful than evolutionary significant unit boundaries for describing the observed genetic structuring in isolated populations of coastal cutthroat trout.

It is important to note, however, that distinguishing between historical associations and ongoing gene flow using Fst can sometimes be problematic (Nielsen and Slatkin 2000). This is particularly relevant for data sets from large, relatively panmictic populations where the effects of genetic drift are weak. In this study, however, Fst can be perceived as a dynamic balance between genetic drift and gene flow because all of these populations were isolated by barriers to upstream fish migration and drift is predicted to be a major factor in populations with small effective population sizes (Neigel 2002).

Regional environmental patterns

Comparisons of genetic and environmental characteristics for Coast Range and Cascade Mountain ecoregions (component and complete sampling strategies) revealed

TABLE 6. Results of two sample *t*-tests comparing mean genetic and environmental variables by sampling strategy and ecoregion. Means and associated P-values (alpha = 0.05) are summarized for total number of alleles for all loci (A), barrier height (Ht), basin area (Area), the ratio of summed tributary length to mainstem stream length (Trib / MS), and the number of steps per basin divided by the average step height in meters (Step Ht).

Sampling Strategy	Ecoregion	n	A	Ht (m)	Area (km^2) (thousands)	Trib/MS (Complexity)	Step Ht (Connectivity)
Western OR	Coast	13	47	19	23.5	0.54	27.3
	Cascade	11	30	7	19.4	0.10	18.7
	P-value	~	0.02	0.15	0.60	0.00	0.02
Component	Coast	8	56	23	32.0	0.68	31.1
	Cascade	8	38	9	26.3	0.11	18.9
	P-value	~	0.01	0.21	0.44	0.01	0.01
Complete	Coast	5	32	12	10.0	0.31	21.4
	Cascade	3	16	3	7.3	0.09	18.4
	P-value	~	0.06	0.29	0.50	0.03	0.46

differences in genetic diversity, channel topology, and within-watershed connectivity (Table 6). Watershed area and barrier heights were not statistically different in all comparisons. The total number of alleles for all loci was higher in the Coast Range in the combined sample ($P = 0.02$) and for component watersheds ($P = 0.01$). When complete watersheds were compared by ecoregion, however, the difference in the average number of alleles was not statistically significant ($P = 0.06$). The differences in within-watershed connectivity was not significant for component basins between the two ecoregions ($P = 0.15$), but was statistically significant for component watersheds and among all western Oregon watersheds ($P < 0.05$).

CHAPTER 4: DISCUSSION

The physical morphology of isolating barriers in this study varied widely, but all were initially judged as persistent barriers to migration based on physical appearance. Genetic analysis supports these observations. All pairwise population comparisons were statistically significant (mean Fst = 0.33, mean alleles for all loci = 5). As expected, populations in this study reflected a higher degree of isolation than has been previously observed among populations of anadromous coastal cutthroat trout (mean Fst = 0.05, mean alleles for 10 loci = 9; Wenburg et al. 2001). Pairwise microsatellite Fst values in this study were comparable to potamodromous westslope cutthroat (*Oncorhynchus clarki lewisi*) in British Columbia (Mean Fst = 0.32, mean alleles for 8 loci = 4; Taylor et al. 2003), but over half of the populations in the Canadian study were not located above physical barriers to migration.

Comprehensive measures of geographic isolation are needed to improve identification and interpretation of factors that control historical and contemporary geographical structure (Sork et al. 1999) at evolutionary meaningful spatial and temporal scales (Faush et al. 2002). Our findings are consistent with recent studies showing the importance of barriers to the structuring of genetic diversity among stream fishes on both local (Carlsson and Nilsson 2001) and regional scales (Taylor et al. 2003; Costello et al. 2003). These studies reveal that the inclusion of barrier-isolated populations in genetic analyses can strongly influence patterns of genetic diversity. Isolated populations can be strongly differentiated because of drift, founder

effects, or repeated genetic bottlenecks (Nei et al. 1975), all of which can act quickly on small populations existing in isolated habitats (Wofford et al. In Press).

Recent attempts to use indices of habitat quality and complexity as predictors of genetic diversity have only been marginally successful. For example, a lack of concordance between habitat size and genetic diversity has been reported for lake populations of brook trout, Salvelinus fontinalis, presumably because of the difficulty in describing the demographic factors responsible for regulating population size (Hebert et al. 2000; Castric et al. 2001). Similarly, physical habitat differences (except migration barriers) among populations of bull trout, Salvelinus confluentus, were not related to patterns of genetic diversity (Costello et al. 2003).

Several factors may contribute to difficulty in identifying and interpreting relationships between environmental features and genetic structure at the watershed scale. A persistent signature of historical events, such as founder effects, may confound contemporary factors in recently colonized habitats where populations may have not yet reached mutation-drift equilibrium (Castric et al. 2001). In addition, populations with large effective population sizes or with easy access for migrants (i.e., high connectivity) may be buffered against the stochastic loss or fixation of alleles from the interaction between landscape structure and demographic processes (Costello et al. 2003).

Our results suggest that strong regional differences in habitat complexity and connectivity are directly associated with differences in levels of genetic diversity. Two factors have increased the probability of detecting these relationships. First, because the Cordilleran Ice did not cover western Oregon, it is likely that coastal

cutthroat populations are closer to mutation-drift equilibrium than fishes in more northerly regions, and therefore, genetic structure is the result of contemporary extinction-recolonization processes (Wade and McCauley 1988; McCauley 1991). Second, because each population is isolated above a substantial waterfall and resides in comparable habitat areas, within-watershed landscape features that influence genetic diversity are easier to identify.

Isolation-by-distance

In this study, isolation-by-distance was statistically significant when all populations were combined. This result may actually reflect the fact that the two ecoregions with different evolutionary forces were analyzed together, rather than the actual physical distance between populations. When the populations in this study were partitioned into ecoregions or evolutionary significant units, isolation-by-distance was not detected in any of the regions. Patterns of scatter among Fst values observed in regional populations suggest that migration was the dominant controlling factor in the Coast Range ecoregion and drift was more prevalent in the Cascade Mountain ecoregion (Fig. 2; Hutchison and Templeton 1999).

Results of this isolation-by-distance analysis are consistent with recent studies. Furthermore, it appears that isolation-by-distance models are too simplistic and should incorporate differences in geographic isolation and permeability to gene flow at multiple spatial scales (Sork et al. 1999). The ability of an organism to disperse across the landscape controls the distribution of genetic diversity (McCauley 1993), and landscape structure has been recognized to influence regional patterns of genetic

structure through dispersal (Keyghobadi et al. 1999; Roland et al. 2000). At the same time, however, interactions between habitat features, stochastic disturbances, and population-level demographic processes may be equally important but have not yet been described.

Disturbance and regional genetic diversity

Disturbance events (e.g., mass wasting) are very common features in headwater landscapes of western Oregon (Swanson and Lienkaemper 1978), and evidence of debris flows of all ages are conspicuous in all of the watersheds that were sampled in the current study. Debris flow can scour stream channels, potentially extirpating or severely reducing populations of stream fishes, especially in headwater habitats (Lamberti et al. 1991; Ensign et al. 1997; Smith et al. 1999; Roghair et al. 2002). In isolated populations, recolonization after a stochastic disturbance is dependent upon the degree of connectivity with adjacent populations.

Strong regional differences in genetic diversity were detected in this study. We hypothesized that watersheds with higher complexity and within-watershed connectivity retain more genetic diversity, in spite of stochastic landscape disturbances. Our results suggested that watersheds in the Coast Range, an area of predominantly sedimentary geology, tended to have more complex drainage patterns and fewer within-watershed obstacles to dispersal than catchments of similar size in the primarily basalt Cascade Mountains (Table 6). Populations in the Coast Range are more likely to retain genetic diversity in the face of stochastic habitat disturbances because there is a low probability that the entire population will be affected by a single

disturbance event (i.e., debris flow). Cascade Mountain populations commonly exist in a single channel with many in-stream barriers to upstream dispersal. In these watersheds, a single debris flow can cause an immediate decrease in genetic diversity followed by a lasting resistance to upstream recolonization and gene flow.

Concomitantly, genetic variation among the populations in this study may have been higher than expected for such extreme physical isolation. One possible explanation may be that catastrophic geomorphic events related to fires or extreme climatic events could rapidly increase the in-channel sediment supply (Gresswell 1999; Dunham et al. 2003; Rieman et al. 2003) and temporarily bury waterfalls. Then for a brief period, fish movement and associated recolonization would facilitate gene flow across barriers over the course of a few years. For example, a typhoon in Japan in 1982 triggered massive land sliding in the Inamata River watershed effectively burying an 11m waterfall until it was hydrologically exhumed in subsequent years (Aniya 1987). Disturbance events such as these are highly stochastic in nature and presumably can occur throughout the study area. Identifying the relative contributions between intermittent gene flow and the lasting effects of landscape structure on genetic diversity remain as an important goal for future research.

Conservation implications

Findings described here may be applicable to a substantial proportion of coastal cutthroat trout. In the steep headwaters of streams, which can comprise up to 80% of the total landscape area, waterfalls are common, and coastal cutthroat trout are often the only fish present. Furthermore, during the process of selecting sampling

locations, it was determined that about 10% (269 watersheds out of 2,678) of the headwater watersheds in western Oregon contained isolated populations of coastal cutthroat trout (Gresswell et al. In Press). In addition, because 24 of the 27 populations were randomly selected from a larger pool of isolated populations, a scope of inference encompassing all of these isolated populations can be assumed.

Although coastal cutthroat trout exist in relatively abundant assemblages of isolated headwater populations, downstream gene flow has not been widely detected, and it is doubtful that there is a substantial contribution of genetic material to downstream populations. An evaluation of fish movement in one of the streams in this study (Camp Creek) suggested negligible movement downstream past a waterfall barrier. Few marked fish (< 1%) were captured in a fish migration trap (screw type) located in the plunge pool immediately below the isolating barrier (Gresswell and Hendricks In Review). Similarly, Michael (1983) did not detect significant movement of barrier-isolated cutthroat trout downstream. These results suggest that it may be unlikely for headwater populations to substantially contribute individuals to declining sea-run stocks existing below the barriers through direct immigration.

On the other hand, the number of migrants may not be the only potential contribution of headwater populations. Small populations encounter strong selection pressures and heritable differences have been detected between above-barrier and below-barrier populations of trout (Northcote and Hartman 1988). Isolated populations have the capacity for developing and preserving novel genotypes that could be lost to drift in populations with larger effective population sizes or that

experience even relatively low levels of gene flow. These novel differences could prove to be adaptive, especially with uncertain future situations (Scudder 1989).

CHAPTER 5: CONCLUSION

In summary, our findings suggest that headwater populations of coastal cutthroat trout above barriers to migration are strongly differentiated. Hierarchical genetic structuring among populations appears to result more from the effect of regional differences in environmental structure than from genetic dispersal following traditional island (Wright 1969) or stepping-stone models (Kimura and Weiss 1964). Genetic diversity was much higher in the Coast Range than in the Cascade Mountains, and this pattern appears to be related to the relative forces of drift and gene flow acting in these different physical environments. Comparisons between landscape structure and genetic diversity suggest that an interaction between stochastic disturbances and physical landscape features may play a substantial role in the structuring of genetic diversity among isolated cutthroat trout populations.

BIBLIOGRAPHY

Angers, B., P. Magnan, M. Plante, and L. Bernatchez. 1999. Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). Molecular Ecology 8:1043-1053

Aniya, M. 1987. Erosion and sedimentation caused by typhoons in 1982 in the Amahata River basin, Japan. Proceedings of the conference on erosion and sedimentation in the Pacific Rim, IAHS publication no. 185, Corvallis, OR 473-482.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tschawytscha*). Journal of Heredity 90:281-288.

Banks, M. A., V. K. Rashbrook, M. J. Calavetta, C. A. Dean, and D. Hedgecock. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. Canadian Journal of Fisheries and Aquatic Sciences 57:915-927.

Bateman, D. S., R. E. Gresswell and C. E. Torgersen. In Review. Evaluating single-pass catch as a tool for identifying spatial pattern in fish distribution. Journal of Freshwater Ecology

Behnke, R. J. 1992. Native Trout of western North America. American Fisheries Society, Bethesda, MD.

Benda, L., and T. Dunne. 1997. Stochastic forcing of sediment supply to channel networks from landsliding and debris flow. Water Resources Research 33:2849-2863.

Bernatchez, L. 1995. A role for molecular systematics in defining evolutionary significant units in fishes. Pp. 114-132 *in* J. L. Nielsen, ed. American Fisheries Society, Bethesda Maryland.

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. Canadian Journal of Fisheries and Aquatic Sciences 44:573-582.

Carlsson, J., K. H. Olsen, J. Nilsson, O. Overli, and O. B. Stabell. 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. Journal of Fish Biology 55:1290-1303..

Carlsson, J., and J. Nilsson. 2001. Effects of geomorphological structures on genetic differentiation among brown trout populations in a Northern Boreal river drainage. Transactions of the American Fisheries Society 130:36-45.

Castric, V., F. Bonney, and L. Bernatchez. 2001. Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. Evolution 55:1016-1028.

Castric, V., and L. Bernatchez. 2003. The rise and fall of isolation by distance in the

anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics 163:983-996.

Costello, A. B., T. E. Down, S. M. Pollard, C. J. Pacas, and E. B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: An examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces : Salmonidae). Evolution 57:328-344.

Currens, K. P., C. B. Schreck, and H. W. Li. 1990. Allozyme and morphological divergence of rainbow-trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. Copeia:730-746.

Dunham, J. B., M. Peacock, C. R. Tracy, J. Nielsen, G. Vinyard. 1999. Assessing extinction risk: integrating genetic information. Conservation Ecology [online] 3(1):2. Available from the internet. URL: http//www.consecol.org/vol3/iss1/art2.

Dunham, J. B., M. K. Young, R. E. Gresswell, and B. E. Rieman. 2003. Effects of fire on fish populations: landscape perspectives on persistence of native fishes and nonnative fish invasions. Forest Ecology and Management 178:183-196.

Ensign, W. E., K. N. Leftwich, P. L. Angermeier, and C. A. Dolloff. 1997. Factors influencing stream fish recovery following a large-scale disturbance. Transactions of the American Fisheries Society 126:895-907.

Fagan, W. F. 2002. Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83:3243-3249.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: Bridging the gap between research and conservation of stream fishes. Bioscience 52:483-498.

Felsenstein, J. 2002. PHYLIP: phylogenetic inference package. Department of Genetics, University of Washington, Seattle, WA

Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A hierarchical framework for stream classification: viewing streams in a watershed context. Environmental Management 10:199-214.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate F-statistics. Journal of Heredity 86:485-486.

Greig, C., D. P. Jacobson, and M. A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology Notes 3:376-379.

Gresswell, R. E. 1999. Fire and aquatic ecosystems in forested biomes of North America. Transactions of the American Fisheries Society 128:193-221.

Gresswell, R. E., D. S. Bateman, G. Lienkaemper, and T. J. Guy. In Press. Geospatial techniques for developing a sampling frame of watersheds across a region *in* T. Nishida, P. J. Kailola, and C. E. Hollingworth, ed. The proceedings of the second international symposium on GIS in fisheries science. Fisheries Research Group, Saitama, Japan.

Gresswell, R. E., and S. R. Hendricks. In Review. Population-scale movement of coastal cutthroat trout in a naturally isolated stream network. Canadian Journal of Fisheries and Aquatic Sciences

Griswold, K. E., K. P. Currens, and G. H. Reeves. 1997. Genetic and meristic divergence of coastal cutthroat trout residing above and below barriers in two coastal basins *in* J. D. Hall, P. A. Bission, and R. E. Gresswell, ed. Sea-run cutthroat trout: biology, management, and future conservation. Oregon Chapter of the American Fisheries Society, Corvallis, OR.

Hansen, M. M., E. E. Nielsen, and K. L. D. Mensberg. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Molecular Ecology 6:469-474.

Hanski, I. 1998. Metapopulation dynamics. Nature 396:41-49.

Hebert, C., R. G. Danzman, M. W. Jones, and L. Bernatchez. 2000. Hydrography and population genetic structure in brook charr (*Salvelinus fontinalis*, Mitchill) from eastern Canada. Molecular Ecology 9:971-982.

Hintze, J. L. 2001. Number Cruncher Statistical Systems, Kaysville, UT.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898-1914.

Keyghobadi, N., J. Roland, and C. Strobeck. 1999. Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). Molecular Ecology 8:1481-1495.

Kimura, M., and G. H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561-576.

Lacy, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1:143-157.

Lamberti, G. A., S. V. Gregory, L. R. Ashkenas, R. C. Wildman, and K. M. S. Moore. 1991. Stream ecosystem recovery following a catastrophic debris flow. Canadian Journal of Fisheries and Aquatic Sciences 48:196-208.

May, C. L., and R. E. Gresswell. 2003. Processes and rates of sediment and wood accumulation in headwater streams of the Oregon Coast Range, USA. Earth Surface Processes and Landforms 28:409-424.

May, C. L., and R. E. Gresswell. 2004. Spatial and temporal patterns of debris-flow deposition in the Oregon Coast Range, USA. Geomorphology 57:135-149.

McCauley, D. E. 1991. Genetic consequences of local population extinction and recolonization. Trends in Ecology and Evolution 6:5-8.

McCauley, D. E. 1993. Genetic consequences of extinction and recolonization in fragmented habitats. Pp. 217-233 *in* P. M. Kareiva, J. G. Kingslover, and R. B. Huey, ed. Biotic interactions and global change. Sinauer Associates, Sunderland, MA.

McPhail, J. D. 1967. Distribution of freshwater fishes in western Washington. Northwest Science 41:1-11.

McPhail, J. D., and C. C. Lindsey. 1986. Zoogeography of the freshwater fishes of Cascadia (the Columbia River and rivers north to the Stikine). Pp. 615-637 *in* C. H. Hocutt, and E. O. Wiley, ed. The Zoogeography of North American Freshwater Fishes. John Wiley and Sons, New York.

Michael, J. H. 1983. Contributions of cutthroat trout in headwater streams to the sea-

run population. California Fish and Game 69:68-76.
Montgomery, D. R., and J. M. Buffington. 1997. Channel-reach morphology in mountain drainage basins. Geological Society of America Bulletin 109:596-611.
Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. Trends in Ecology and Evolution 9:373-375.
Morris, D. B., K. R. Richard, and J. M. Wright. 1996. Polymorphic microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 53:120-126.
Nehlsen, W., J. E. Williams, and L. A. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 16:4-21.
Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.
Neigel, J. E. 2002. Is F-ST obsolete? Conservation Genetics 3:167-173.
Nielsen, J. L., and G. K. Sage. 2002. Population genetic structure in Lahontan cutthroat trout. Transactions of the American Fisheries Society 131:376-388.
Nielsen, R., and M. Slatkin. 2000. Likelihood analysis of ongoing gene flow and historical association. Evolution 54:44-50.
Northcote, T. G., and G. F. Hartman. 1988. The biology and significance of stream trout populations (*Salmo spp.*) living above and below waterfalls. Polskie Archiwum Hydrobiologii 35:409-442.
Olsen, J. B., J. K. Wenburg, and P. Bentzen. 1996. Semiautomated multilocus genotyping of Pacific salmon (Oncorhynchus spp) using microsatellites. Molecular Marine Biology and Biotechnology 5:259-272.
Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.
Reeves, G. H., L.E. Benda, K.M. Burnett, P.A. Bisson, and J.R. Sedell. 1995. A disturbance-based ecosystem approach to maintaining and restoring freshwater habitats of evolutionarily significant units of anadromous salmonids in the Pacific Northwest *in* J. L. Nielsen, ed. Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society Symposium 17, Bethesda, Maryland.
Reeves, G. H., P. A. Bisson, and J. M. Dambacher. 1998. Fish Communities *in* R. J. Naiman, R. E. Bilby, ed. River Ecology and Management. Springer- Verlag, New York, NY.
Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.
Rieman, B. E., C. H. Luce, R. E. Gresswell, and M. K. Young. 2003. Introduction to the effects of wildland fire on aquatic ecosystems in the Western USA. Forest Ecology and Management 178:1-3.
Roghair, C. N., C. A. Dolloff, and M. K. Underwood. 2002. Response of a brook trout population and in stream habitat to a catastrophic flood and debris flow. Transactions of the American Fisheries Society 131:718-730.
Roland, J., N. Keyghobadi, and S. Fownes. 2000. Alpine *Parnassius* butterfly dispersal: Effects of landscape and population size. Ecology 81:1642-1653.

Schneider, S., J. -M. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin version 1: an exploratory population genetics software environment. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Smith, P. P., and J. B. Atkinson. 1999. Flood and debris-flow effects on Virginia brook trout populations. Proceedings Annual Conference Southeast Association of Fish and Wildlife Agencies 53:91-99.

Sork, V. L., J. Nason, D. R. Campbell, and J. F. Fernandez. 1999. Landscape approaches to historical and contemporary gene flow in plants. Trends in Ecology & Evolution 14:219-224.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies. Journal of Animal Ecology 46:337-365.

Swanson, F. J., G. W. Lienkaemper. 1978. Physical consequences of large organic debris in Pacific Northwest streams. USDA Forest Service, General Technical Report. Pacific Northwest Forest and Range Experiment Station, PNW-69.

Swanson, F. J., L. E. Benda, S. H. Duncan, G. E. Grant, W. F. Megahan, L. M. Reid, and R.R. Ziemer. 1987. Mass failures and other processes of sediment production in Pacific Northwest forest landscapes. Pp. 9-38 *in* E. O. Salo, and T. W. Cundy, ed. Streamside management: forestry and fishery interactions. University of Washington, Institute of Forest Resources, Contribution No. 57.

Taylor, E. B., Z. Redenbach, A. B. Costello, S. M. Pollard, and C. J. Pacas. 2001. Nested analysis of genetic diversity in northwestern North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*). Canadian Journal of Fisheries and Aquatic Sciences 58:406-420.

Taylor, E. B., M. D. Stamford, and J. S. Baxter. 2003. Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. Molecular Ecology 12:2609-2622.

Taylor, P. W., and S. D. Roberts. 1999. Clove oil: An alternative anesthetic for aquaculture. North American Journal of Aquaculture 61:150-155.

Trotter, P. C. 1989. Coastal cutthroat trout: a life history compendium. Transactions of the American Fisheries Society 118:463-473.

Trotter, P. C., P. A. Bisson, and B. R. Fransen. 1993. Status and plight of the searun cutthroat trout *in* J. G. Cloud, and G. H. Thorgaard, ed. Genetic conservation of salmonid fishes, Plenum, New York.

Wade, M. J., and D. E. McCauley. 1988. The effects of extinction and colonization on the genetic differentiation of populations. Evolution 42:995-1005.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus spp.* and the definition of a species under the Endangered Species Act. Marine Fisheries Review 53:11-22.

Wenburg, J. K., and P. Bentzen. 2001. Genetic and behavioral evidence for restricted gene flow among coastal cutthroat trout populations. Transactions of the American Fisheries Society 130:1049-1069.

White, P. S., and S. T. A. Pickett. 1985. Natural disturbance and patch dynamics: an introduction *in* S. T. A. Pickett, P. S. White, ed. The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, Florida.

Wier, B. S., C. C. Cockerham. 1984. Estimating F-statistics for the analysis of

population structure. Evolution 38:1358-1370.
Wofford, J. E. B., R.E. Gresswell and M.A. Banks. In Press. Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. Ecological Applications
Wright, S. 1969. The theory of gene frequencies, Vol 2. University of Chicago Press
Wydoski, R. S., and R. R. Whitney. 1979. Inland fishes of Washington. University of Washington Press, Seattle, WA.

APPENDICES

TABLE A1. Thermocycler conditions and reagents used for each multiplex.

Multiplex*	Markers	Reagents MgCl2 (mM)	Reagents dNTPs (mM)	Thermocycler conditions
Set A	One102	0.4000	0.1000	94°C for 180s
	One103			12 cycles at 94°C for 60s+ 49°C for 30s + 72°C for 15s
	One108			15 cycles at 94°C for 30s + 49°C for 30s + 72°C for 15s
				94°C for 30s
				49°C for 30s
				72°C for 300s
Set B	Omy77	0.0240	0.1000	92°C for 300s
	Ots4			25 cycles at 92°C for 30s + 56°C for 30s + 72°C for 30s
				72°C for 1800s
Set C	Ots209	0.4000	0.0625	94°C for 180s
	Ots212			32 cycles at 94°C for 30s + 63°C for 20s + 72°C for 30s
				72°C for 120s

*All PCR reactions were conducted in 5µl volumes using 1µl DNA template, 0.25µl enhancer mix (830µl H20, 20µl Tween, 50µl BSA (20mg/ml)), 100µl formamide, and 5µl Tris/KCL buffer (Set A,B) or PromegaÒ 10x buffer (Set C).

TABLE A2. Genetic summary for coastal cutthroat trout at 7 loci. The number of alleles (A), allelic size range (R), expected heterozygosity (He), observed heterozygosity, and number of sucessful geotypes (N) are tabulated for each population and locus.

		Locus							
Creek		One102	One103	One108	Omy77	Ots4	Ots209	Ots212	Mean
gle	A	10	8	11	12	5	7	11	9
	R	200-252	114-154	154-266	103-139	109-125	139-167	107-159	~
	He	0.85	0.73	0.72	0.86	0.71	0.80	0.63	0.76
	Ho	0.82	0.69	0.64	0.83	0.62	0.76	0.65	0.72
	N	79	94	84	90	87	76	83	85
roy	A	11	8	11	11	5	8	7	9
	R	200-252	114-154	150-246	107-141	109-125	139-167	107-159	~
	He	0.81	0.73	0.70	0.81	0.70	0.73	0.71	0.74
	Ho	0.82	0.62	0.72	0.75	0.70	0.81	0.64	0.72
	N	65	77	76	88	88	74	74	77
brf	A	7	3	10	11	4	4	6	6
	R	196-244	114-146	150-238	107-147	109-119	147-159	107-139	~
	He	0.70	0.63	0.85	0.87	0.59	0.68	0.79	0.73
	Ho	0.64	0.62	0.77	0.84	0.60	0.71	0.69	0.69
	N	67	71	65	80	82	68	68	72
dea	A	3	3	5	5	2	3	6	4
	R	200-224	130-142	162-266	109-153	119-121	155-163	107-155	~
	He	0.52	0.60	0.68	0.63	0.50	0.29	0.77	0.57
	Ho	0.52	0.59	0.67	0.47	0.55	0.27	0.79	0.55
	N	81	82	81	76	76	81	84	80
efl	A	11	8	9	10	7	6	6	8
	R	200-244	114-146	138-242	121-161	107-125	143-163	107-143	~
	He	0.85	0.84	0.85	0.78	0.58	0.80	0.17	0.69
	Ho	0.82	0.83	0.91	0.78	0.56	0.79	0.17	0.70
	N	78	77	80	85	87	91	92	84
wol	A	2	5	2	2	2	4	2	3
	R	252-256	122-158	150	107-127	119-125	139-167	107-135	~
	He	0.33	0.38	0.50	0.43	0.41	0.39	0.48	0.42
	Ho	0.27	0.39	0.64	0.45	0.35	0.33	0.79	0.46
	N	55	64	66	84	83	70	92	73
cav	A	7	6	10	9	5	7	8	7
	R	200-248	114-166	146	107-143	107-129	139-167	107-171	~
	He	0.49	0.59	0.84	0.76	0.62	0.76	0.81	0.70
	Ho	0.53	0.60	0.81	0.63	0.47	0.69	0.81	0.65
	N	73	85	72	70	62	72	78	73
luk	A	14	7	7	8	4	4	8	7
	R	192-256	106-146	138-194	125-153	107-125	151-163	103-179	~
	He	0.78	0.72	0.80	0.70	0.46	0.62	0.78	0.69
	Ho	0.78	0.78	0.71	0.70	0.48	0.61	0.81	0.69
	N	80	90	86	76	87	77	89	84
eco	A	10	10	8	13	6	7	11	9
	R	200-268	114-154	154-238	103-141	109-125	139-167	107-159	~
	He	0.82	0.86	0.80	0.87	0.69	0.69	0.81	0.79
	Ho	0.83	0.89	0.65	0.73	0.69	0.56	0.86	0.74
	N	88	90	89	51	48	77	84	75
tuc	A	7	7	10	13	6	7	10	9
	R	200-236	114-154	150-258	103-141	109-125	139-167	107-159	~
	He	0.79	0.82	0.69	0.88	0.69	0.71	0.81	0.77
	Ho	0.65	0.73	0.49	0.76	0.67	0.59	0.88	0.68
	N	65	83	69	89	88	82	84	80
mul	A	5	6	8	8	4	3	6	6
	R	200-240	114-50	150-250	107-157	107-121	147-169	107-135	~
	He	0.66	0.76	0.76	0.74	0.44	0.40	0.79	0.65
	Ho	0.66	0.70	0.59	0.61	0.38	0.37	0.82	0.59
	N	70	84	74	82	78	70	71	76
swe	A	11	8	10	10	5	6	7	8
	R	196-248	114-162	154-242	107-169	109-125	139-167	107-139	~
	He	0.83	0.66	0.83	0.83	0.65	0.63	0.84	0.75