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THE COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*): GENETIC POPULATION STRUCTURE, MIGRATION PATTERNS AND LIFE HISTORY TRAITS

by

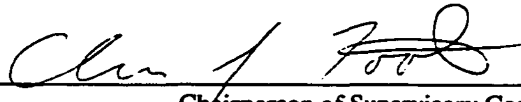
John Keim Wenburg

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

University of Washington

1998

Approved by 
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Program Authorized to Offer Degree School of Fisheries

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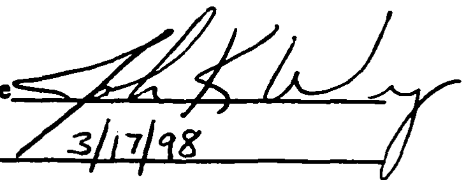

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University of Washington

Abstract

THE COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*): GENETIC POPULATION STRUCTURE, MIGRATION PATTERNS AND LIFE HISTORY TRAITS

by John Keim Wenburg

Chairperson of the Supervisory Committee:
Assistant Professor Chris J. Foote
Department of Fisheries

Coastal cutthroat trout (*Oncorhynchus clarki clarki*) populations have undergone major range wide declines over the past two decades. This study was initiated in light of their declining status in an effort to augment the paucity of existing data, both in terms of their general ecology and genetic population structuring. Multiplexed groups of fluorescently labeled microsatellite primers were used in an analyses of allelic variation, both on a statewide and microgeographic scale. Analysis of six loci for 13 anadromous populations from throughout Washington revealed high within population variability (H_E = 71%; mean # alleles/locus = 24), significant differences in genotypic frequencies for single-locus pairwise comparisons between all populations, and substantial population subdivision ($F_{ST} = 0.121$, $R_{ST} = 0.093$). Similarly, analysis of allelic variation at 10 loci for 10 populations from within Hood Canal revealed high within population variability (H_E = 69%; mean # alleles/locus = 17), significant differences in genotypic frequencies between all populations across all loci, and in 304 of 450 single-locus pairwise comparisons, and significant population subdivision ($F_{ST} = 0.030$, $R_{ST} = 0.029$); all of

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which suggest that individual creeks form the basis for distinct breeding units in this subspecies. Mantel tests supported an isolation by distance model of population structure within Hood Canal using both F_{ST} (P=0.015) and the Cavalli-Sforza and Edwards' (1967) chord distance (P=0.001) as measures of genetic distance, and for the latter on a statewide scale (P=0.01). Estimated levels of gene flow from direct observations, through a 3-year tag-recapture study, were similar to those estimated indirectly from allele frequency data, being on average approximately 5 to 10 effective migrants per generation between neighboring populations. Intensive ecological study of the Big Beef Creek population (Hood Canal, WA) revealed: differing migration patterns between the sexes, with males migrating into freshwater sooner and remaining longer than females; significant correlation between the upstream migration date for individuals across years (R^2 = 0.43, P=0.002); and evidence that some individuals likely overwintered at sea. Survival estimates averaged 54% for the overwintering period in freshwater, and 12-24% and 33-59% during salt water migrations for first and second-time migrants, respectively.

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DEDICATION

The author wishes to dedicate this dissertation to his family, for their love and continued support in all his endeavors.

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INTRODUCTION

The coastal cutthroat trout (*Oncorhynchus clarki clarki*) is the most widely distributed of 14 currently recognized cutthroat subspecies, exhibiting a wide array of complex life history strategies and highly variable migration patterns (Behnke 1992). Its range extends from the Eel River in California, USA, to Gore Point on the Kenai Peninsula in Alaska, USA, with inland penetration generally limited to less than 150 km (Behnke 1992). Coastal cutthroat trout have received little study relative to other more economically important, commercially harvested Pacific salmonids, and as such, there is a relative paucity of genetic and ecological information available for coastal cutthroat trout.

Coastal cutthroat trout have undergone a major range-wide decline over the past two decades (Nehlsen et al. 1991; Trotter et al. 1993), although data are limited and locally some populations remain healthy. The American Fisheries Society Endangered Species Committee concluded that all native naturally spawning populations in the states of California, Oregon and Washington are at some level of risk, being either of special concern or on the threshold of becoming threatened or endangered (Nehlsen et al. 1991). The California Department of Fish and Game has classified the subspecies as a "Species of Special Concern" (Gerstung 1997), in Oregon they are currently listed on the

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"Sensitive Species List" (Hooton 1997), and in Washington, although there appears to be a mix of healthy and depressed populations, many populations are in a state of steady decline (Leider 1997). In addition, the United States National Marine Fisheries Service (NMFS) recently listed all forms of the coastal cutthroat trout of the North Umpqua River in Oregon as endangered pursuant to the Endangered Species Act of 1973 (ESA) (NMFS 1996).

This study was designed to address some of the shortcomings in the available information on coastal cutthroat trout; specifically, in three main areas: genetic population structure, migration patterns and life history traits. This was achieved through a combination of tracking of individual coastal cutthroat trout migrants in and among four creeks in Hood Canal, Washington and genetic analysis of these and several other populations from throughout the state of Washington, the results of which are reported in the following five chapters. Detailed background information on coastal cutthroat trout biology along with the questions raised and hypotheses tested are given in each chapter. The chapters are intended to be complete on their own, however where appropriate, they are cross-referenced to highlight the connectivity between them. Brief descriptions of the specific contents for each of the chapters follow.

Chapters 1 and 2 report various methodological developments which were necessary for the completion of the subsequent chapters. Specifically, Chapter 1 highlights the development of a novel tagging method used throughout the field portion of this study. Placement of visible implant (VI) tags in the anal fin tissue allowed the

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tracking of individually identifiable coastal cutthroat trout migrants for up to three years as they migrated into and out of fresh water. This chapter was previously published in an almost identical form, and is published again here with permission from the American Fisheries Society:

Wenburg JK, George GW (1995) Placement of visible implant tags in the anal fin of wild coastal cutthroat trout. North American Journal of Fisheries Management, 15, 874-877.

Chapter 2 details the development of the genetic techniques used throughout this study. Microsatellite primer fluorescent labeling technology was used to develop several groups of multiplexed microsatellite loci that allowed for relatively fast acquisition of allele frequency information in coastal cutthroat trout and steelhead (*Oncorhynchus mykiss*). This chapter reports the details of their development and highlights their usefulness through the analysis of three populations each, for both coastal cutthroat trout and steelhead. This chapter was also previously published in an almost identical form, and is published again here with permission from Blackwell Science, Inc.:

Wenburg JK, Olsen JB, Bentzen P (1996) Multiplexed systems of microsatellites for genetic analysis in coastal cutthroat trout (*Oncorhynchus clarki clarki*) and steelhead (*Oncorhynchus mykiss*). Molecular Marine Biology and Biotechnology, 5, 273-283.

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This paper was published along with a companion paper that is included here as Appendix A, again with permission from Blackwell Science, Inc.:

Olsen JB, Wenburg JK, Bentzen P (1996) Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. Molecular Marine Biology and Biotechnology, 5, 259-272.

Chapter 3 details a genetic investigation of coastal cutthroat trout populations from throughout the state of Washington. Sample acquisition for this portion of the study was performed in collaboration with the Washington Department of Fish and Wildlife (WDFW). Specifically, this chapter reports on the results from the analysis of six microsatellite loci for 13 anadromous populations and one Yellowstone cutthroat (*Oncorhynchus clarki bouvieri*) population, which was used as an outgroup (raw data given in Appendix B, D and E). This chapter was also previously published in almost identical form, and is published again here with permission from Blackwell Science, Inc.:

Wenburg JK, Bentzen P, Foote CJ (1998) Microsatellite analysis of genetic variation in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Molecular Ecology, *In press*.

Chapter 4 combines the analysis of allele frequency data for 10 microsatellite loci from 10 coastal cutthroat trout populations with physical migrational pattern information in an analysis of gene flow dynamics on a microgeographic scale in Hood Canal,

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Washington (raw data given in Appendix C and E). Further, the genetic data from Chapter 3 was integrated to provide an overall synthesis of coastal cutthroat trout population structure on various geographic scales. In addition, these data were used to investigate various issues involving the properties and mutational dynamics exhibited by microsatellite loci.

Chapter 5 details the results of an ecological tag-recapture and scale analysis study, which focused on the most intensely studied population, Big Beef Creek (scale analysis results given in Appendix F, and data for individual recaptures given in Appendix G). The University of Washington, in conjunction with the WDFW, maintains a permanent weir facility located on Big Beef Creek, which empties directly into Hood Canal from the Kitsap Peninsula, Washington. Big Beef Creek is a low gradient watershed (lower than 400 m with gradient ranges from 0.5-1.5%) containing 18 km of main stream channel with a basin area of approximately 38 km^2. Big Beef Creek contains a wild native population of anadromous coastal cutthroat trout. Periodic planting of various strains of hatchery coastal cutthroat trout have occurred in Big Beef Creek over the last several decades. However, throughout Hood Canal, plantings were sporadic and have not occurred since the 1980's due to poor returns and likely had little affect on the genetic makeup of this population. Various survival estimates are reported in this chapter along with details of habitat usage and results from tests for various hypothesis on differential migration patterns and life history traits between male and female coastal cutthroat trout.

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CHAPTER 1: PLACEMENT OF VISIBLE IMPLANT TAGS IN THE ANAL FIN OF WILD COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*)

ABSTRACT

Typical periocular (adipose eyelid) placement of visible implant tags proved inadequate for tagging of wild coastal cutthroat trout *Oncorhynchus clarki clarki* because of individual variation in periocular tissue, excessive stress of tagging procedures, and the inability to consistently tag fish smaller than 150 mm fork length (FL). A novel technique for placement was developed which alleviated these problems. Tags were placed in the clear tissue between rays of the anal fin. Migrating smolts and adults 110-451 mm FL were successfully tagged in this manner. Adults recaptured 4-85 d after tag placement ($N = 59$) exhibited 98% retention, with all but two tags readable *in situ*. Smolts held in stream enclosures 24 h after tagging ($N = 120$) exhibited 97% tag retention with all tags readable *in situ*. Inspection of insertion points revealed significant tissue healing which rendered future tag losses unlikely.

INTRODUCTION

Individual identification of living fish is necessary for many fisheries studies. Most available tagging methods providing individual identification involve external tags (e.g., transbody, spaghetti, Petersen disc, anchor) which have significant disadvantages (Nielsen 1992). External tags often have low retention rates (Buckley and Blankenship

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1990; McFarlane et al. 1990); may impede swimming ability, and may increase metabolic costs, mortality, and predation (Nielsen 1992). These drawbacks are often not acceptable in ecological research, especially when populations of threatened or endangered fishes are being studied.

Visible implant (VI) tags (Northwest Marine Technology, Shaw Island, Washington), are internal tags that can be read externally, minimizing many of the drawbacks of traditional external tags. Visible implant tags are made of biocompatible plastic and are printed with a three-digit alpha-numeric code. Tags are available in two sizes in each of six colors and are designed to be placed in transparent tissues. Tags are inserted with a single-shot hypodermic syringe with a flattened push rod (Blankenship and Tipping 1993). Haw et al. (1990) reported the development and initial testing of VI tags placed in transparent periocular tissue (adipose eyelid) of hatchery strains of rainbow trout (*Oncorhynchus mykiss*). Blankenship and Tipping (1993) evaluated this technique more intensively in a hatchery strain of coastal cutthroat trout (*O. clarki clarki*). Although this method is becoming common in hatchery tagging operations for several salmonid species, it proved to be unsuitable here.

The amount of periocular tissue present was found to be extremely variable in wild coastal cutthroat trout. Periocular tissue apparently varies between wild and hatchery cutthroat trout, being less developed in the former. Others have reported variation in periocular tissue between strains of rainbow trout (Haw et al. 1990) and between full sibling groups in hatchery Chinook salmon *O. tshawytscha* (J. Hooff,

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Washington Department of Fish and Wildlife, personal communication). In this study, although a small percentage of wild cutthroat were easily tagged with traditional placement, the majority did not possess adequate periocular tissue, thus proving difficult to tag with this standard technique.

When periocular tag placement was attempted, injectors had to be extremely sharp and fish had to be heavily anesthetized, as any movement could cause tearing of the periocular tissue and make tagging impossible. In addition, the extreme amounts of sedation necessary and the possibility of associated increases in handling mortality were of concern, as was the potential for injury to fish when manipulating sharp instruments near the eye. Even under heavy sedation, periocular tissue was often inadequate for tagging fish of all size ranges (110 - 451 mm, all measurements given are fork length unless otherwise noted), most notably for fish smaller than 150 mm, especially under field conditions. Successful periocular tagging rates ranged from 10-50% in preliminary tests. Others have reported that periocular placement is ineffective for salmonids less than 150 mm (P. Bergman, Northwest Marine Technology, personal communication): Haw et al. (1990) reported tagging of fish larger than 149 mm, Blankenship and Tipping (1993) used fish greater than 200 mm, and Bryan and Ney (1994) reported retention rates of only 50% in wild brook trout *Salvelinus fontinalis* less than 160 mm total length.

Because of the problems outlined above, periocular insertion was rejected for tagging of wild coastal cutthroat trout. In ecological research, especially that involving threatened or endangered species, all possible efforts must be made to use noninvasive

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techniques. Such ethical considerations are too often lacking in modern ecological research, and explicit discussion of them is almost non-existent (Farnsworth and Rosovsky 1993). In striving to find a reliable, noninvasive method of individually tagging wild coastal cutthroat trout (110-451 mm), a novel technique was developed for placing visible implant tags between the third to sixth rays of the anal fin.

METHODS

Standard size VI tags (2.5 mm long, 0.08 mm thick) printed in white on a black background were used in this study. Best results were achieved by lightly anesthetizing fish with MS-222 (tricaine: 1 g powder / 20 L water) and placing them on a board with their belly along the length of a raised lip (2 cm) on the bottom edge. The anal fin was then spread out with one finger along the flat surface of the lip for tagging. Addition of a cloth or rubber flap on one side of the board, under which the fish's head was placed, aided in immobilizing the fish and reducing stress. Initial insertion of the injector was between rays of the anal fin at approximately two-thirds of the fin length away from the body, with final tag placement near the half-way point (Figure 1.1). The injector was slowly inserted towards the body of the fish, with care taken to prevent piercing the entire fin. This was accomplished by initially inserting the injector at a 25° angle and flattening out the injector to a nearly horizontal position once the top layer of tissue was pierced. When the tissue had covered the entire opening of the injector, the tag was pushed in as far as possible before injector removal. If placement was unsuccessful, or the tissue was torn, another site was selected between an adjacent set of fin rays. After insertion of the

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tag, any air in the insertion point was pinched out, firmly seating the tag. Tags were placed just under the surface of the tissue in larger fish (> 250 mm) to facilitate reading. This method provided rapid and reliable identification of large numbers of fish at a relatively low cost (US $0.85/tag).

This method was used to tag 125 adults, 210-451 mm (mean = 357; SD = 42) and 544 smolts, 95-262 mm (mean = 153; SD = 31) at Big Beef Creek in central Hood Canal, Washington. A permanent weir at this site allowed for year-round capture of migrant cutthroat trout. Adults were captured entering or leaving fresh water from December 29, 1993, to June 1, 1994. To determine retention rates, each VI-tagged fish was double-marked with a small upper caudal fin clip. It was assumed that these marks were retained throughout the study because growth during the winter months is minimal for cutthroat, exemplified by the fact that fin clips were easily recognized on all trout that were recaptured with VI tags in place. Smolts were captured leaving fresh water from March 25 to June 1, 1994. The retention rates of VI tags in smolts were evaluated by holding a subsample of fish for 24 h in stream enclosures.

Anal fin tissue did not vary among wild cutthroat trout, rendering all fish of a given size equally taggable. The anal fin method was easily applied to cutthroat trout 125 mm or larger, with fish as small as 110 mm being taggable, but less reliably. Risk of serious injury to fish during implantation was lessened with anal fin placement, because anal fin tissue will regenerate if torn or punctured, whereas eye tissue will not. Tagging in adipose, pectoral and ventral fins was not effective because placement was difficult

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and natural marks often made tags difficult to detect and read. For example, tag placement in the adipose fin, as suggested by Haw et al. (1990), proved difficult in small fish, and tag visibility was extremely limited by the natural coloration and spots on the adipose fins of coastal cutthroat trout. Similarly, although placement of tags in the dorsal fin was easy to do and would likely prove to be extremely secure (because tags are inserted from above), natural spotting rendered tags difficult or impossible to read in this location. However, placement of VI tags in other fins warrants further study and may be useful for other species.

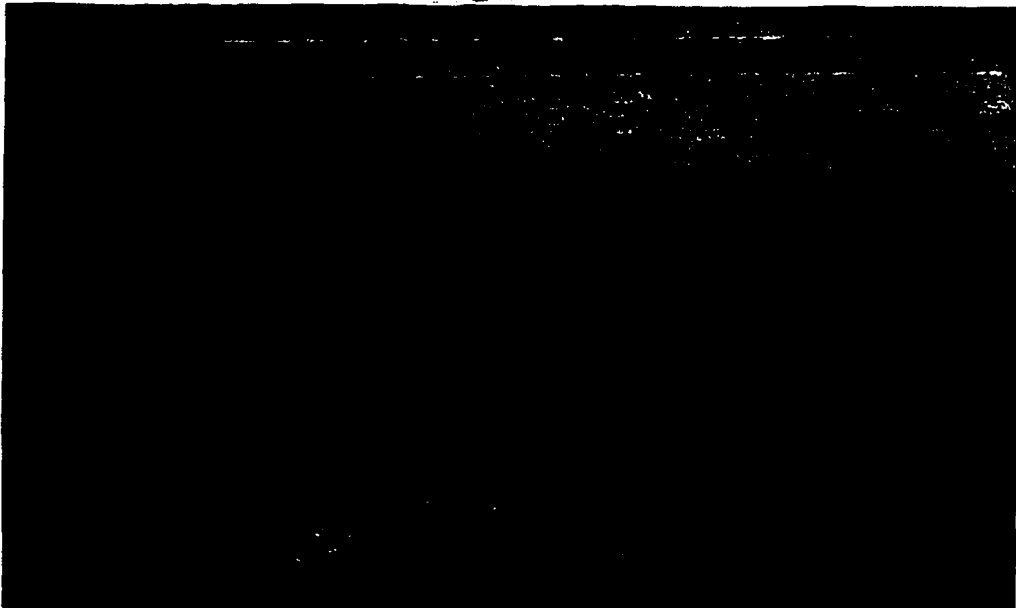

Figure 1.1 Visible implant tag (number C14 on black background) in the anal fin of a wild adult coastal cutthroat trout.

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RETENTION RATES

Tags were retained in 59 of 60 adults (98%) migrating to and from fresh water (4-85 d). Some fish were captured multiple times while migrating back and forth between fresh water and salt water, but were counted only once in calculating tag retention. Two tags were unreadable in large males with particularly dark anal fins, characteristic of the spawning run. However, the presence of tags in these fish was obvious. These tags were easily extracted with a small incision and reinserted closer to the surface of the tissue between adjacent rays. This tagging procedure did not increase adult mortality. Survival rates were actually higher for tagged fish (46%; N=125) than for those not tagged (38%; N=63).

Retention rates were high even after trout spawned. Of the 59 surviving adult migrants retaining the anal fin tag, 55 (93%) were determined to have spawned between initial tagging and recapture, based on visual assessment and comparison of condition factors (K = weight in g/fork length in mm^3) at each point. Spawning behavior, especially nest-digging by females, provided a strong testimony to security of anal fin tags. In contrast, internal passive integrated transponder (PIT) tags yielded retention rates of only 65% in spawning fish (N = 23), with fish presumably extruding this tag with their eggs or sperm.

Smolt retention rates over a 24-h period were 97% (116 of 120) in fish ranging from 110-250 mm (mean = 155; SD = 24). Fish that lost tags during this period were

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139, 153, 166, and 180 mm, suggesting that tag retention was independent of length throughout the size range tagged. Smolt mortality during this period was zero. Blankenship and Tipping (1993) found long-term retention rates (3-21 months) to be high (94%) with periocular placement. Similar results are expected for longer term retention rates for smolts tagged in the anal fin. Examination of anal fin tags 24 h after placement revealed firmly seated tags with insertion wounds grown over, rendering future tag loss unlikely.

DISCUSSION

Tagging with VI tags had previously been shown to be an effective method for identifying individuals, eliminating the disadvantages of traditional external tags (Nielsen 1992). Typical VI tag placement in the periocular tissue provides relatively low-cost, externally readable tags. These tags have no external component which may hamper swimming, become tangled in debris or pull loose, and tags are inconspicuous enough to prevent them from being keyed upon by predators. As a complementary procedure, the method of anal fin placement described here was easier to perform, required less sedation of the fish, was less likely to cause serious harm, and could be performed on fish as small as 110 mm. In addition, the amount of suitable anal fin tissue did not vary critically among individuals of a given size as did periocular tissue, and overall retention rates were high. Anal fin placement of VI tags proved to be a valuable technique for the tagging of coastal cutthroat trout and is likely applicable to many species, providing a relatively noninvasive tagging method for fisheries research.

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CHAPTER 2: MULTIPLEXED SYSTEMS OF MICROSATELLITES FOR GENETIC ANALYSIS IN COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*) AND STEELHEAD (*ONCORHYNCHUS MYKISS*)

ABSTRACT

This study reports on the first application of microsatellites to genetic surveys of the endangered coastal cutthroat trout (*Oncorhynchus clarki clarki*). In addition, the application of microsatellites derived from other species to steelhead/rainbow trout (*O. mykiss*) is described, thus extending the suite of microsatellite markers available for this species. Thirty-five microsatellite primer sets were screened, previously developed from six salmonid species, to assess their value as genetic markers for cutthroat and steelhead; of these, 21 primer sets yielded high-quality amplification products in both species. Ten primer sets were used to develop four species-specific multiplex sets, containing six and three loci for cutthroat, and six and four loci for steelhead, and applied to three populations of each species. Each of the four sets were amplified in a single polymerase chain reaction (PCR) using fluorescently labeled primers, visualized in a single gel lane, and analyzed using a semi-automated multilocus genotyping system. Numbers of alleles per locus averaged 12 in cutthroat and 8 in steelhead populations. Observed heterozygosities were 0.26-0.97 in cutthroat and 0.00-0.92 in steelhead, (mean = 0.70 and 0.54, respectively). Significant differences in allele frequencies were found in 54 of

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57 pairwise interpopulation comparisons in the two species. The probabilities of match (P_M) for the 9-locus and 10-locus systems were very low in each of the three populations surveyed in cutthroat and steelhead (1.2×10^{-16} to 4.5×10^{-15} and 4.2×10^{-15} to 6.2×10^{-8}, respectively). In all but one population, the average polymorphic information content (PIC) per locus was greater than 0.5, and therefore considered highly informative. These multiplex methods are reproducible, resolve extensive genetic variation, and are compatible with non-lethal sampling; hence, they should prove useful for a wide range of genetic investigations involving coastal cutthroat trout and steelhead.

INTRODUCTION

Coastal cutthroat trout (*Oncorhynchus clarki clarki*) have undergone a major decline throughout their range from California to Prince William Sound in Alaska over the past 15 to 20 years (Trotter et al. 1993). Nehlsen et al. (1991) listed 13 stocks of coastal cutthroat trout as being at risk of extinction, the Endangered Species Committee of the American Fisheries Society identified all populations of coastal cutthroat trout in Washington, Oregon and California as being at some level of risk of extinction, and the National Marine Fisheries Service (NMFS) has recently ruled that the North Umpqua coastal cutthroat trout warrants listing as an endangered species pursuant to the Endangered Species Act of 1973 (for convenience "cutthroat" will hereafter refer to the coastal/anadromous subspecies of cutthroat, *O. clarki clarki*, unless otherwise noted).

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As with any threatened or endangered species, identifying distinct intraspecific population units is crucial to the development of comprehensive management plans (Waples et al. 1990; Utter et al. 1993). To date, only one significant genetic study has been reported for coastal cutthroat trout. This study analyzed allozyme variation in populations from Puget Sound, Washington, and suggested that they were genetically nested by region, and likely distinct at the tributary creek level (Campton and Utter 1987). It is important that more genetic studies be performed on this subspecies throughout its native range to positively identify distinct population units.

DNA analysis can be performed from extremely small amounts of tissue, such as a small fin clips, and therefore does not necessitate the sacrifice of organisms as do most allozyme studies. In light of the endangered status of coastal cutthroat trout, noninvasive DNA analysis provides a more conservative and ecologically responsible approach for genetic analysis than does further allozyme study. Microsatellites are one class of highly polymorphic DNA markers which are being increasingly used for a range of applications in fisheries and aquaculture research (see references in Olsen et al. 1996, Appendix A). The high variability and Mendelian inheritance exhibited by microsatellite loci make them amenable to population differentiation studies, parentage determination in mixed family groups, and even individual fingerprint "tagging". Recent interest in microsatellites for these and other applications has led to the development of primers from a variety of salmonids (see Appendix A in Appendix A).

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To date, there have been no microsatellite loci cloned from or applied to genetic analysis in cutthroat trout. However, at many microsatellite loci, the priming sequences flanking the tandem repeat motifs themselves are conserved among related taxa (e.g, Morris et al. 1996; Scribner et al. 1996). Therefore, microsatellite primers derived from one species may be used to amplify homologous loci in related species or genera. This cross-specificity may eliminate the need for time-consuming and expensive development of primer sequences from each species of interest, and makes possible evolutionary comparisons of repeat sizes and variability among species (although such comparisons require sequencing of amplified products to verify homology of loci and conservation of repeat structures). Here the possibility of applying microsatellite primers developed from other salmonids to coastal cutthroat trout and steelhead (*O. mykiss*) was explored.

Steelhead and cutthroat are closely related, often occur sympatrically and are morphologically very similar (Campton and Utter 1985; Trotter 1989; Phillips and Pleyte 1991). Fry, juveniles and even smolts of these species can be very difficult to identify to the species level and natural hybridization is known to occur between them (Campton and Utter 1985). The main focus here was the identification of microsatellite markers for cutthroat research; however, given the broad sympatry, close genetic relationship, and occurrence of hybridization between the two species, both species were surveyed with the same markers. Species markers based on allozymes have been identified for cutthroat/steelhead discrimination (Campton and Utter 1985), however, diagnostic markers are not yet available for DNA analysis. The potential use of microsatellites as

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species markers will be reported elsewhere. In a broader context, identifying suites of microsatellites that can be assayed in both species serves to lay the groundwork for future studies of genetic differentiation between the two species in areas where they do or do not hybridize. Finally, this approach augments the set of microsatellite markers presently available for steelhead/rainbow (Nielsen et al. 1994, Sakamoto et al. 1994; Morris et al. 1996).

To date, microsatellite studies have been constrained by several factors including their relatively slow and costly data collection in comparison with allozyme analyses (see also Olsen et al. 1996, Appendix A). To increase efficiency, it is possible to co-amplify several microsatellite loci in a single tube polymerase chain reaction (PCR), referred to as a multiplex PCR (Paetkau et al. 1995; Urquhart et al. 1995; O'Reilly et al. 1996; Olsen et al. 1996, Appendix A). In addition, in-lane fluorescent size standards and fluorescent labeling of primers, used in conjunction with automated genotyping detection systems (see details in Olsen et al. 1996, Appendix A) can further improve efficiency of data collection by permitting the scoring of multiplexed loci with overlapping allelic size ranges in a single gel lane. These techniques can produce analytical systems with extremely high throughput and sensitive discrimination. Such analyses potentially approach traditional allozyme studies in efficiency, and typically exceed allozymes in assayable genetic variation. This study reports on the development of such multiplex systems using fluorescently labeled microsatellite primers, which, along with a companion study by Olsen et. al. (1996, Appendix A), provides the first applications of

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this technology to salmonid research and the first DNA study of any kind in coastal cutthroat trout.

The specific objectives of this study were as follows: 1) to determine which of 35 microsatellite loci (see Table 2.1, see also Appendix A in Appendix A) could be reliably amplified and scored in coastal cutthroat trout and steelhead; 2) to develop multiplexed groups of fluorescently labeled versions of these loci to increase the speed and efficiency of genetic surveys; and 3) to analyze three populations from each species using the microsatellite systems developed to quantify the amount of variability assayable at these loci and to assess their applicability to various genetic investigations.

RESULTS

SCREENING AND MULTIPLEXING

Table 2.1 summarizes the results of the unlabeled microsatellite screening for cutthroat and steelhead at all 35 loci. For each locus, optimum annealing temperatures, size range of products and product quality scores are given. Although in many cases a wide range of annealing temperatures yielded acceptable results, those given in Table 2.1 provided the best results for each locus. In almost all cases, lower temperatures could be used, but product quality tended to decline as annealing temperatures decreased below the optimum. The annealing temperatures were not always increased to the point where no product was produced; hence, in some cases product quality may be improved through the use of higher annealing temperatures. For the eight loci that yielded no amplification

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product (score of 5) the annealing temperature given is the lowest attempted. Further modifications of annealing temperature or magnesium concentrations (or both) might have yielded products. Such modifications were not employed as the goal was to identify suites of loci that amplify well under uniform and relatively stringent conditions. Of the 35 loci screened in each species, 21 were given quality codes of 1 or 2, and as such are potentially useful for a range of genetic applications in these species.

Table 2.1 Microsatellite screening summary.

Microsatellite Locus[a]	Cutthroat (T)-(Q)-(S)	Steelhead (T)-(Q)-(S)
Fgt1	(60)-3-2	(60)-2-3
Omy77	**(56)-2-1,2**	**(50)-2-1,2**
Omy78	(58)-4-1	(58)-4-1
Omy87	(60)-2-1,2	(60)-3-1,2
Omy207	(60)-2-2,3	(60)-2-1,2
Omy293	(55)-4-1	(55)-4-1
Omy325	**(60)-2-1,2**	**(60)-2-1,2**
Oneμ1	(58)-1-1,2	(58)-1-1,2
Oneμ2	**(52)-2-3,4**	**(52)-2-3,4**
Oneμ8	**(52)-2-3,4**	**(52)-2-3,4**
Oneμ10	(56)-3-1,2	(56)-3-1,2
Oneμ11	**(56)-1-2**	**(56)-1-2**

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Table 2.1 continued

Oneμ14	**(56)-2-2-5**	**(56)-2-2**
Ots1	**(56)-2-3,4**	**(56)-2-2-4**
Ots2	(45)-5	(45)-5
Ots3	**(52)-1-1**	**(52)-1-1,2**
Ots4	**(52)-1-1**	**(52)-1-1**
Ots5	(45)-5	(45)-5
Ots6[b]	**(60)-1-3**	**(60)-1-3**
PuPuPy	(60)-1-5	(60)-1-5
Sfo8	**(56)-2-3**	**(56)-2-3,4**
Sfo12	(55)-5	(55)-5
Sfo18	(65)-5	(65)-5
Sfo23	(60)-5	(60)-5
Ssa4	(60)-2-2,3	(60)-2-2,3
Ssa14	**(52)-2-2**	**(52)-2-2**
Ssa85	**(56)-2-1,2**	**(56)-2-1,2**
Ssa171[b]	**(56)-1-1**	**(56)-1-1**
Ssa197	(58)-1-1	(58)-1-1,2
Ssa202	(58)-5	(58)-5
Ssa289	(60)-5	(60)-5
Ssa293	(55)-3-1,2	(55)-3-1,2
μSat15	(55)-5	(55)-5
μSat60	(56)-3-1,2	(56)-3-1,2
μSat7[b]	**(60)-1-2**	**(60)-1-2**

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Table 2.1 continued

Total Screened	35	35
Score 1-2	21	21
Score 3-4	6	6
Score 5	8	8

Amplification results are coded as follows: (T) is the PCR annealing temperature; (Q) indicates the quality of the PCR amplification and (S) indicates the approximate size range (bases) of the PCR product. The loci used in multiplex development are shown in bold. Code for product quality (after Pepin et al. 1995): 1, amplification of one or two bands and no stutter; 2, amplification of one or two bands and some stutter; 3, multiple bands and no smearing; 4, multiple bands and smearing; 5, no amplification. Code for allelic size range: 1, 60-120 b; 2, 120-180 b; 3, 180-240 b; 4, 240-300 b; 5, >300 b.

[a]Prefix Codes: Fgt, Omy and PuPuPy (*Oncorhynchus mykiss*); Oneμ (*O. nerka*); Ots (*O. tshawytscha*); Sfo (*Salvelinus fontinalis*); Ssa (*Salmo salar*); μSat (*S. trutta*).

[b]Monomorphic in all fish tested.

Using a subset of the 15 primer pairs that yielded the highest-quality PCR products two multiplex sets were optimized for each species. Table 2.2 summarizes the fluorescent tag designations, primer concentrations, and the annealing temperatures for the multiplex groups developed. Two of the 15 pairs, μSat73 and Ots6, were found to be fixed for a single allele in both species and therefore were not included in multiplex sets.

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Ssa171 was fixed for a single allele in cutthroat and appeared to amplify two monomorphic loci in steelhead, as two bands appeared in all individuals. Therefore, this primer pair was also discarded for multiplex development, but is currently being used in an ongoing species marker study. Although Ots3 yielded high quality products when amplified and visualized alone, it was discarded as its products were often too small (< 75 b) to be scored on a consistent basis when included in multiplex groupings. In gels containing multiplexed amplifications, fluorescent primers and small artifactual PCR products tended to overlay such small alleles, rendering them unreadable. Ots4 provided high quality products when amplified alone, but appeared to inhibit amplification of other loci when multiplexed, and was also excluded from multiplex development. Finally, Oneμ8 was used in steelhead multiplex set B but failed to amplify reliably in cutthroat and therefore was not included in any cutthroat sets (this locus accounts for the overall difference in the number of steelhead (10) and cutthroat loci (9) in multiplex sets). The remaining nine loci were included in the multiplex sets for both species.

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Table 2.2 Multiplex set composition. Fluorescent label assignment[a], PCR annealing temperature, and primer concentrations for each multiplex set are given.

		Microsatellite loci and primer concentration (µM)		
Multiplex set label	Anneal °C	Fam	Hex	Tet
Cutthroat A	56	Oneµ11 (0.1)	Omy77 (0.3)	Ssa85 (0.15)
		Sfo8 (0.2)	Oneµ14 (0.5)	Ots1 (0.4)
Cutthroat B	52	Oneµ2 (0.06)	Omy325 (.15)	Ssa14 (0.55)
Steelhead A	56	Oneµ11 (0.06)	Omy77 (0.15)	Ssa85 (0.06)
		Sfo8 (0.1)	Oneµ14 (0.14)	Ots1 (0.17)
Steelhead B	52	Oneµ2 (0.055)	Omy325 (.11)	Ssa14 (0.55)
			Oneµ8 (0.13)	

[a]Fam=6-carboxyfluorescein, Hex=hexachloro-6-carboxyfluorescein, Tet=tetrachloro-6-carboxyfluorescein. The fluorescent labels appear as blue, yellow and green, respectively, using ABI filter set B.

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Within each multiplex set, alleles were identified based on the combination of size range and fluorescent color label (see Figure 2 in Olsen et al. 1996, Appendix A). All 9 or 10 loci, depending on species, were amplified and scored for each individual using only two PCRs and two gel lanes. It is possible to run two gels in a typical day on the ABI 373A, each with 36 lanes. Therefore, these systems allow one researcher to run up to 36 samples per day at 9 or 10 loci, or alternatively 72 samples per day at 3, 4 or 6 loci, depending on the multiplex sets used.

Two tests confirmed the precision and reproducibility of genotype scores. First, PCRs, gel electrophoresis and allele scoring were repeated at least once in six or more individuals for each locus in the multiplex sets. In all cases allele scores varied by less than 0.5 bases (b) in repeat analyses of the same individual, and in most by less than 0.1 b (see Table 4 in Olsen et al. 1996, Appendix A), effectively yielding identical allele designations. Second, two families of each species were screened at each of the 15 loci evaluated for multiplex development. In all cases, every allele exhibited by the offspring was present in one or both parents (Figure 2.1). Although families were too small to statistically assess inheritance patterns, allele distributions were consistent with expected segregation ratios, further confirming genotype reproducibility and the expected Mendelian inheritance of PCR amplification products.

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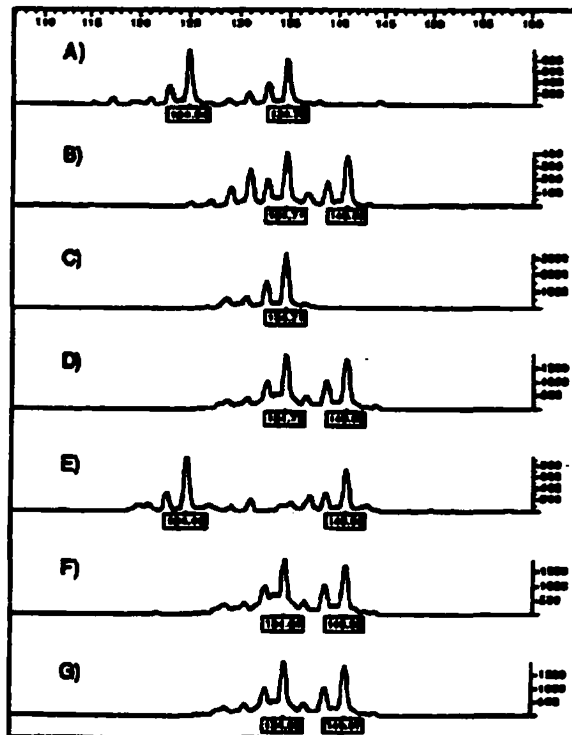

Figure 2.1 Electropherogram results from a coastal cutthroat trout family at microsatellite locus Omy77 consisting of a mother (A), father (B), and five offspring (C-G). Note that each offspring allele is attributable to one or both parents. Two families were screened for each species at all 15 loci evaluated for multiplex development. Scales on the horizontal and vertical axis denote estimated fragment size and relative fluorescent units, respectively.

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Population Screening and Data Analysis

The 9 and 10-loci multiplexed surveys from three populations of cutthroat and steelhead revealed extensive genetic variation (Table 2.3). The average number of alleles per locus ranged from 10 to 13 in the three cutthroat populations and from 5 to 13 in the three steelhead populations. Variation in the sizes of alleles at individual loci ranged from 2 to 172 b (mean = 61 b) in cutthroat, and ranged from 0 to 113 b (mean = 28 b) in steelhead. There were rare occurrences of allelic overlap between loci with the same fluorescent label in two of the four multiplex sets (Ssa85 and Ots1 in cutthroat set A; Omy77 and Oneμ14 in steelhead set A). In most of these cases, electropherogram peak profiles revealed unique amplification signatures for each locus, allowing overlapping alleles from various loci to be distinguished (Figure 2.2). For the few cases were this was not possible, a single locus PCR for one of the overlapping loci was performed with visualization in an independent gel lane. Comparison of these results with the original multilocus results allowed unambiguous assignment of all alleles.

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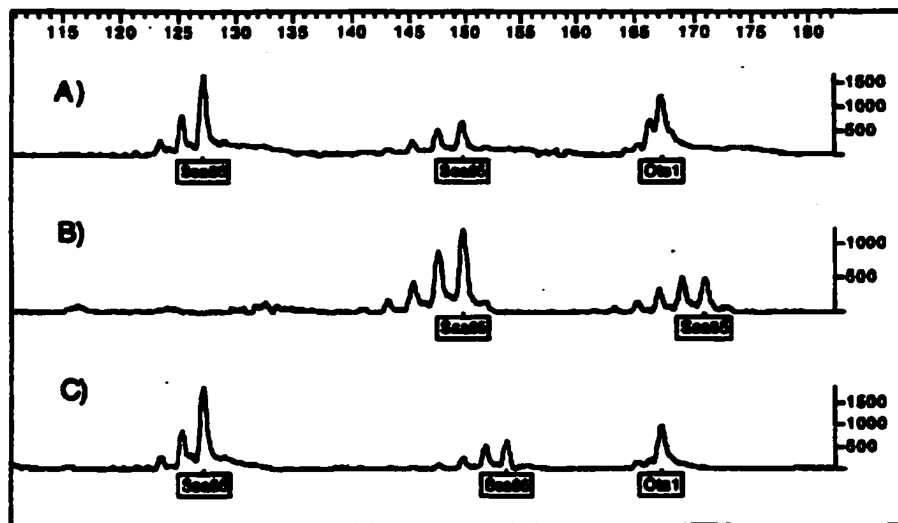

Figure 2.2 Example of overlapping alleles for loci labeled with the same fluorescent color. Note the qualitative variation in peak "signature" for Ssa85 and Ots1 alleles. Signatures remained consistent across samples and could often be relied upon to distinguish between alleles falling within the size range overlap, as shown here between 160-170 b. Scales on the horizontal and vertical axes denote estimated fragment size and relative fluorescent units, respectively.

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Table 2.3 Summary statistics for microsatellite loci by population. "A" indicates number of alleles; "R" allele size range; "*N*" sample size; "C" frequency of the most common allele with size in parentheses; "PIC" polymorphic information content; and "P_M," match probability for the most frequent genotype.

Pop.[a]/Stat.	Omy77	Oneμ11	Oneμ14	Ots1	Sfo8	Ssa85	Omy325	Oneμ2	Ssa14	Oneμ8	Average[b]
<u>Cutthroat1</u>											
A	11	2	16	16	13	17	15	9	7	--	12
R	113-145	146-148	218-322	239-305	192-240	111-197	99-177	212-232	123-135	--	--
N	31	33	33	33	33	33	31	31	32	--	32
C	.23(127)	.70(146)	.32(278)	.20(271)	.39(212)	.27(153)	.13(163)	.29(228)	.61(127)	--	0.35
PIC	0.835	0.333	0.848	0.875	0.764	0.869	0.902	0.817	0.559	--	0.756
P_M	0.017	0.265	0.015	0.008	0.045	0.015	0.004	0.017	0.191	--	2.5×10^{-14}
<u>Cutthroat2</u>											
A	15	3	23	12	12	16	19	12	7	--	13
R	111-145	144-148	155-322	167-295	194-277	109-171	99-177	212-234	121-135	--	--
N	49	42	47	48	49	49	49	48	49	--	48
C	.20(123)	.48(146)	.19(290)	.19(273)	.22(212)	.26(153)	.19(99)	.28(228)	.57(127)	--	0.29
PIC	0.874	0.466	0.914	0.870	0.828	0.862	0.871	0.816	0.577	--	0.786
P_M	0.010	0.128	0.011	0.007	0.007	0.007	0.015	0.011	0.150	--	7.5×10^{-16}

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Table 2.3 continued

Cutthroat3											
A	10	3	13	16	10	9	11	13	8	--	10
R	109-141	144-148	218-318	167-293	192-228	125-161	105-177	208-280	119-141	--	--
N	59	59	57	60	60	59	59	59	60	--	59
C	.28(129)	.54(148)	.46(242)	.18(277)	.24(214)	.31(161)	.25(153)	.38(274)	.40(139)	--	0.34
PIC	0.808	0.464	0.736	0.885	0.843	0.789	0.829	0.786	0.678	--	0.758
P_M	0.007	0.259	0.069	0.007	0.014	0.049	0.014	0.010	0.054	--	$8.6x10^{-14}$
Steelhead1											
A	11	2	4	10	19	8	20	23	10	9	13
R	117-137	144-146	147-163	159-251	228-315	105-135	95-151	237-350	129-155	150-178	--
N	55	53	55	51	54	53	58	53	58	55	55
C	.28(130)	.74(146)	.63(159)	.25(177)	.38(263)	.58(104)	.21(109)	.11(251)	.50(141)	.26(174)	0.39
PIC	0.789	0.308	0.432	0.821	0.814	0.594	0.894	0.934	0.625	0.810	0.780
P_M	0.040	0.260	0.190	0.014	0.049	0.129	0.007	0.003	0.096	0.012	$4.1x10^{-12}$
Steelhead2											
A	fixed	2	4	2	10	6	9	14	5	3	6
R	153	144-148	149-159	153-161	246-303	105-135	99-145	243-283	129-133	152-170	--

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Table 2.3 continued

N	36	41	41	41	34	41	37	41	41	40	39
C	1.00	.98(144)	.50(159)	.85(159)	.35(291)	.82(130)	.41(101)	.33(256)	.83(131)	.54(160)	0.66
PIC	0.000	0.046	0.563	0.219	0.745	0.306	0.739	0.798	0.271	0.564	0.472
P_M	1.000	0.905	0.086"	0.500	0.031	0.466	0.059	0.029	0.101	0.640	9.7×10^{-07}
Steelhead3											
A	fixed	fixed	5	3	6	4	9	11	4	5	5
R	153	144.00	151-161	153-161	273-293	127-135	97-145	245-279	115-133	152-170	--
N	18	21	21	20	15	21	21	21	21	20	20
C	1.00	1.00	.43(159)	.80(159)	.43(279)	.76(130)	.26(101)	.29(253)	.75(131)	.52(160)	0.62
PIC	0.000	0.000	0.644	0.314	0.677	0.368	0.805	0.823	0.389	0.535	0.506
P_M	1.000	1.000	0.057	0.360	0.111	0.383	0.036	0.020	0.227	0.423	6.4×10^{-07}

[a] *Cutthroat1* indicates Bear Creek; *Cutthroat2*, Seabeck Creek; *Cutthroat3*, Gierin Creek; *Steelhead1*, Selway R.; *Steelhead2*, Kavachina R.; *Steelhead3*, Snotolvayam R.

[b] Average P_M is the match probability multiplied across all loci. All other values are true averages.

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To further assess the relative utility of each locus for applications to specific genetic studies of cutthroat and steelhead, two descriptive index parameters were estimated, PIC and P_M (Table 2.3). PIC values provide a measure of the informativeness of a locus as applied to kinship, parentage and gene mapping studies, with values 0.5 or above being considered highly informative (Botstein et al. 1980). Average PIC for the Kavachina River steelhead population was 0.472, while the values for all other populations ranged from 0.506 to 0.786, and were higher, on average, in cutthroat. P_M values, which are a conservative estimate of the probability of choosing two unrelated individuals from a population with identical genotypes, ranged from 1.2×10^{-16} to 6.2×10^{-8} across the populations surveyed.

The population survey results are shown in Tables 2.4 and 2.5. Table 2.4 summarizes the observed and expected heterozygosities for all locus-population combinations. Observed heterozygosities ranged from 0.00 in three fixed steelhead locus-population combinations to 0.97 at Oneμ2 in the Bear Creek cutthroat population. The average heterozygosity for all locus-population combinations was 0.70 for cutthroat and 0.54 for steelhead. In 4 of 24 cases, significant heterozygote deficiencies were observed in cutthroat populations (initial $\alpha = 0.017$), although no locus had significant departures in all populations. In 7 of 30 steelhead locus-population comparisons, significant heterozygote deficiencies were observed (initial $\alpha = 0.017$), but again no locus exhibited them in all populations. Table 2.5 summarizes χ^2 pairwise tests of

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independence between populations at each locus. Significant differences were found in 52 of 57 comparisons (initial $\alpha = 0.0055$ for cutthroat, 0.005 for steelhead).

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Table 2.4 Heterozygosities by population. "H_O" observed heterozygosity; and "H_E" expected heterozygosity are given for each population-locus combination.

Population[a]	Stat.	Omy77	Oneμ11	Oneμ14[b]	Ots1	Sfo8	Ssa85	Omy325	Oneμ2	Oneμ8	Ssa14	Avg./pop
Cutthroat1	H_O	0.84	0.36	--	0.91	0.79	0.91	0.87	0.97	--	0.53	0.71
	H_E	0.87	0.43	--	0.90	0.80	0.89	0.92	0.85	--	0.60	
Cutthroat2	H_O	0.90	0.26*	--	0.88	0.86	0.82	0.96	0.77	--	0.49*	0.69
	H_E	0.89	0.57	--	0.89	0.85	0.88	0.89	0.84	--	0.62	
Cutthroat3	H_O	0.75*	0.66	--	0.92	0.88	0.81	0.88	0.88	--	0.62*	0.71
	H_E	0.84	0.56	--	0.90	0.87	0.82	0.85	0.81	--	0.73	
Steelhead1	H_O	0.87	0.47	0.53	0.82	0.74*	0.57	0.86*	0.92*	0.72*	0.71	0.72
	H_E	0.82	0.38	0.51	0.85	0.83	0.63	0.91	0.95	0.84	0.67	
Steelhead2	H_O	0.00	0.05	0.76	0.29	0.68	0.32	0.59*	0.63*	0.68	0.05*	0.41

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Table 2.4 continued

	H_E	--	0.05	0.64	0.25	0.79	0.32	0.78	0.83	0.63	0.30	
Steelhead3	H_O	0.00	0.00	0.81	0.40	0.73	0.29	0.90	0.76	0.76	0.30	0.50
	H_E	--	--	0.71	0.35	0.74	0.41	0.85	0.86	0.62	0.43	

[a]Population designations as in Table 2.3.

[b] Heterozygosities are not reported for cutthroat populations for this locus as alleles were binned by groups of four bases for scoring, causing heterozygosities to be artificially decreased.

*Values judged to indicate significant heterozygote deficiencies based on Hardy-Weinberg equilibrium following sequential Bonferroni adjustment (initial $\alpha = 0.017$).

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Table 2.5 Pairwise tests of allelic independence between populations.

Population[a]		Omy77	Oneμ11	Oneμ14	Ots1	Sfo8	Ssa85	Omy325	Oneμ2	Ssa14	Oneμ8
Cutthroat1 x Cutthroat2											
	p	0.002*	0.004*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.003*	0.041*	--
	S.E.	0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	0.008	--
Cutthroat1 x Cutthroat3											
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	--
	S.E.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	--
Cutthroat2 x Cutthroat3											
	p	<0.001*	0.405	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	--
	S.E.	<0.001	0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	--
Steelhead1 x Steelhead2											
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	S.E.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Steelhead1 x Steelhead3											

Table 2.5 continued

p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
S.E.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Steelhead2 x Steelhead3										
p	<0.001*	0.544	0.083	<0.001*	0.001*	0.819	<0.001*	<0.001*	0.015	<0.001*
S.E.	<0.001	0.005	0.010	<0.001	0.001	0.011	<0.001	<0.001	0.003	<0.001

[a] Population designations as in Table 2.3.

*Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.0055$ for cutthroat populations and 0.005 for steelhead).

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Unpurified DNA extracts obtained using a quick lysis DNA protocol provided suitable DNA for microsatellite amplification in all populations with the exception of Gierin Creek cutthroat. Initial attempts to use DNA from the quick lysis protocol for this population yielded unacceptable results. It appeared that the process of freezing and freezing and thawing of tissues before immersion in EtOH had a degenerative effect on the quality of the DNA. This was also found this to be true with tissue from other populations (data not shown) which were frozen and thawed one or more times before extraction. Phenol/chloroform DNA extraction of the Gierin Creek tissue samples did yield acceptable results; however, these samples still proved to be more recalcitrant to amplification than quick lysis extracts from the other populations where tissue was taken from live fish and immediately placed in EtOH. In future sampling efforts, when the possibility exists to either take tissue from live fish in the field or take tissue from frozen fish at a later date, investigators may want to consider these findings.

DISCUSSION

This study represents the first genetic investigation of coastal cutthroat trout using microsatellites. The application of heterologous microsatellite primers to steelhead was also demonstrated. As in a companion study by Olsen et al. (1996, Appendix A), the success in application of microsatellites to these species, with primers developed from other salmonids, demonstrates that interspecific and even intergeneric exchange of primers may be used in lieu of or in addition to development of species-specific primers.

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Therefore, at least in salmonids, for which a relatively large number of microsatellite primers are available, expensive and time-consuming cloning of microsatellites and the subsequent development of new primers may not be necessary for many genetic applications. As demonstrated here, at least 21 of the 35 markers screened may be used successfully in cutthroat and steelhead. Further, at these loci, allele designations were consistent, apparently heritable, and reproducible and genetic variation was extensive.

Any attempts at multiplexing more than three loci (one for each available fluorescent color) must rely on size separation between similarly labeled products, making allelic size ranges the major constraining factor in determining the maximum number of multiplexed loci that can be visualized in a single gel lane. The average allelic size range exhibited here for cutthroat (61 b) is among the highest reported for any fish or mammal species, surpassed only by Atlantic cod (*Gadus morhua*) which averaged 71 b across six loci (see O'Reilly et al. 1996, and references therein), making multiplexing of six or more loci relatively difficult. Nonetheless, as knowledge of allelic size ranges for particular species-locus combinations increases, so too will the possible number of multiplexed loci. The results obtained here and by Olsen et al. (1996, Appendix A) suggest that salmonid multiplex systems of at least nine loci are feasible.

The few cases of heterozygote deficiency found were not prevalent enough to strongly suggest the occurrence of null alleles at any of the loci examined (see discussion in Olsen et al. 1996, Appendix A). Where deficiencies did occur, they were not present in all populations of a species and are most likely indicative of population substructuring

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or a sampling bias. For instance, the population sample that exhibited the largest number of heterozygote deficits was the Selway River which was the only artificially reared population used in this study. This population consisted of offspring from a spawning of only 18 females and seven males, which were unexpectedly found to be an admixture of separate hatchery and wild populations, most likely causing the deficiencies observed.

Pairwise tests of independence for populations of each species conformed to expectations based on their geographic proximity (Table 2.5). Bear Creek and Seabeck Creek are separated by over 50 km of shoreline in Hood Canal; Gierin Creek, located in the Strait of Juan de Fuca, is separated from both by large expanses of open water, which act as an effective barriers to cutthroat migration (Trotter 1989). As expected, all cutthroat populations were found to be independent from each other at the loci tested here. The two Russian steelhead populations were from rivers that meet in tidewater, and hence were expected to be much more closely related to each other than either was to the Selway River (Idaho) population. In fact, the Selway River population differed significantly from both Russian populations at all loci, whereas the latter two differed significantly from each other at only 6 of the 10 loci tested.

Use of the multiplexed groups of fluorescently labeled microsatellite primers developed here hold the potential for substantial gains in speed and efficiency of genetic data collection, relative to manual methods of single-locus analysis. Up to six loci were co-amplified in a single tube PCR, the products of which were unambiguously identified in a single gel lane. As such, one researcher, using the ABI373A automated sequencer

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was able to collect up to 432 single-locus genotypes per day. This approaches, and possibly exceeds, protein allozyme studies in efficiency of data collection and cost. This fact, along with the threatened status of coastal cutthroat trout, and the non-lethality of tissue sampling, point to microsatellite analysis as an attractive alternative to allozyme analysis for further genetic studies of depleted populations.

The multiplex systems reported here, and modifications thereof, have a number of potential applications. The extremely low P_M and correspondingly high PIC and heterozygosity values for most of these loci indicate that even applications necessitating high levels of discrimination, such as kinship and parentage studies, are feasible.

This study provides markers and methods for efficient nonlethal genetic investigation of the endangered coastal cutthroat trout subspecies. It also identifies additional markers to augment existing microsatellites usable for steelhead. Along with the companion study by Olsen et al. (1996, Appendix A), the results given here confirm that microsatellite analysis can be a relatively fast and cost-effective investigative technique, applicable to a variety of genetic issues in fisheries and conservation.

EXPERIMENTAL PROCEDURES

DNA Extraction

Small caudal fin clips (approx. $0.5cm^2$) were taken from live fish for two cutthroat populations, Bear Creek ($N = 33$) (*Cutthroat1*), and Seabeck Creek ($N = 50$)

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(*Cutthroat2*), from Hood Canal, Washington, and three steelhead populations, Selway River ($N = 58$) (*Steelhead1*), Idaho, and Kavachina River ($N = 41$) (*Steelhead2*) and Snotolvayam River ($N = 21$) (*Steelhead3*), Kamchatka Peninsula, Russia. Clips were placed immediately in vials containing 95% EtOH and stored at ambient temperature for one day to two years before DNA extraction. DNA was released from approximately one quarter to half of each clip using a quick lysis method (details in Olsen et al. 1996, Appendix A). DNA extracts were stored at -20°C for up to 12 months, and thawed at room temperature immediately preceding PCR. Samples for the third cutthroat population, Gierin Creek ($N = 60$) (*Cutthroat3*), Washington, were obtained from the Washington Department of Fish and Wildlife (WDFW) from individuals which were being lethally sampled for another study. Fish were placed on dry ice in the field and transferred to -80°C freezers within 12 hours. One to six months later, caudal or pelvic fin clips were taken and placed in 95% EtOH. One to 10 weeks later, approximately 25 mg of tissue was subjected to standard phenol/chloroform DNA extraction methods (Hoelzel and Green 1992), resuspended in 100 µL of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), stored at -20°C for up to three months, and thawed at room temperature immediately preceding PCR.

SCREENING AND MULTIPLEXING OF LOCI

Two rounds of microsatellite screening were completed in this study. The first was performed using unlabeled microsatellite primers to determine which loci were amplifiable in cutthroat and steelhead, and which were most promising for multiplex

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development. Loci producing the highest quality amplification products were subjected to a second round of screening using fluorescently labeled versions of the primer pairs, and a subset of these were further used in multiplex group development. All microsatellite primers, labeled and unlabeled, were synthesized on a Beckman Oligo 1000 DNA synthesizer or purchased from Keystone Laboratories (Menlo Park, Calif., U.S.A.) (see Appendix A in Appendix A, for a complete list of primer sequences). For initial tests, annealing temperatures, PCR temperature profiles, and reaction conditions were based on those reported by the developers of each microsatellite locus. Typically, the recommended annealing temperatures were reduced by 3° to 5° in initial screening to accommodate for possible variations in repeat flanking regions. Temperatures and primer concentrations were then incrementally increased or decreased where appropriate to produce the cleanest possible products. To maintain the highest possible level of reproducibility (see Olsen et al. 1996, Appendix A), standard PCR profiles and reaction conditions were used for all cutthroat and steelhead microsatellite screening, with only the annealing temperature and primer concentrations being varied. The standard PCR profile was one cycle of 94°C for 180 sec; 10 cycles of 94°C for 60 s + X °C for 30 sec + 72°C for 15 sec + 14 cycles of 94°C for 30 sec + X °C for 30 sec + 72°C for 15 sec; and one cycle of 94°C for 30 sec + X °C for 30 sec + 72°C for 300 sec, where X was the loci-specific annealing temperature. Amplifications were carried out in 10mM TrisHCl (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.8mM dNTP's (0.2mM each), 0.4 units *Taq* polymerase, 1 µL crude DNA extract from quick lysis samples or 100-250 ng purified

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DNA from phenol/chloroform extractions, 0.04-0.60 µM forward and reverse primer, and dH_20 to yield a final volume of 10 µL.

For unlabeled primer reactions, the amplification products were electrophoresed on standard 6% acrylamide gels and stained with SYBR Green (Molecular Probes Inc.) before being scanned and visualized on a Molecular Dynamics FluorImager 575 (details in Olsen et al. 1996, Appendix A). Product quality was then visually scored on a scale of 1-5 (Table 2.1) (see Figure 1 in Olsen et al. 1996, Appendix A, for examples of FluorImager gel images used to assess PCR product quality). For the 21 loci scoring 1 or 2, fluorescently labeled versions of the primers were synthesized and subjected to a second round of screening and optimization.

Fluorescently labeled primer reaction products were electrophoresed on a 6% denaturing polyacrylamide gel on a Perkin-Elmer Applied Biosystems Inc. (ABI) 373A automated sequencer using GeneScan 672 analysis software, ver. 1.1 (ABI, 1993) (details in Olsen et al. 1996, Appendix A). Amplification products were scored for quality using the output electropherograms generated by Genotyper software, version 1.1 (ABI, 1994) and quality codes analogous to those used for the unlabeled primer products above (see Figure 1 in Olsen et al. 1996, Appendix A, for examples of Genotyper electropherogram images used to assess PCR product quality), and subjected to similar optimization as were unlabeled primers (details in Olsen et al. 1996, Appendix A).

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Of the 21 labeled primers screened, the 15 most promising (bold-faced type in Table 2.1) were selected for further use in multiplex group development. Initially, sets of three primers, one for each available fluorescent label color, were formed for each species. By repeatedly varying annealing temperatures and primer concentrations (details in Olsen et al. 1996, Appendix A), it was possible to increase the size of the multiplex sets up to a maximum of six by duplicating colors for loci determined to have non-overlapping allelic size ranges.

Population Screening and Data Analysis

Three cutthroat and three steelhead populations were genotyped at 9 and 10 microsatellite loci, respectively, using the multiplexing PCR sets developed here (Table 2.2). For scoring, each allele peak was selected manually in the Genotyper program which then automatically estimated the product size in bases (b). Category templates were constructed in Genotyper allowing automated binning of alleles in various size and color categories, dictated by the user. For all loci except Oneμ14 in cutthroat, categories were determined by rounding allele designations to the nearest base and forming a bin of +/- 1 b around them (all loci in this study were dinucleotide repeats). Ladder-like peak profiles (referred to as "stutter", see Olsen et al. 1996, Appendix A) of the electropherogram results for Oneμ14 in cutthroat made exact determination of allele sizes unreliable for heterozygotes differing by only one repeat (2 b). Therefore, as a conservative measure, bins of +/- 2 b were used for this locus which had the effect of reducing the occurrence of heterozygote detection.

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Two methods were employed to assess accuracy and reproducibility of allele scoring, which has been called into question for microsatellite loci (see Olsen et al. 1996, Appendix A). First, PCR, electrophoresis, and allele scoring were repeated for at least six individuals at each locus. Second, two families of each species consisting of a mother, father and five offspring were scored at each locus.

Allele size data was summarized and analyzed using the GENEPOP version 1.1 computer program (Raymond and Rousset 1995). With this program, tests for conformity to Hardy-Weinberg equilibrium were performed for each locus-population combination using the algorithms of Louis and Dempster (1987) and Guo and Thompson (1992), and estimates of independence between three populations within each species were made at each locus following the χ^2 analysis of independence according to Raymond and Rousset (1995). Sequential Bonferroni adjustments (Rice 1989) were used throughout this study to judge significance levels for simultaneous tests with an initial α level of 0.05. The polymorphic information content (PIC) was computed for every locus in each population following Botstein et al. (1980). The match probability (P_M) was calculated for every locus-population combination independently by squaring the frequency of the most common genotype, and for multilocus genotypes by multiplying P_M values across all loci for each population (Edwards et al. 1992).

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CHAPTER 3: MICROSATELLITE ANALYSIS OF GENETIC POPULATION STRUCTURE IN AN ENDANGERED SALMONID: THE COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*)

ABSTRACT

The genetic population structure of coastal cutthroat trout (*Oncorhynchus clarki clarki*) in Washington state was investigated by analysis of variation in allele frequencies at six highly polymorphic microsatellite loci for 13 anadromous populations, along with one outgroup population from the Yellowstone subspecies (*O. clarki bouvieri)* (mean heterozygosity = 67%; average number of alleles per locus = 24). Tests for genetic differentiation revealed highly significant differences in genotypic frequencies for pairwise comparisons between all populations within geographic regions and overall population subdivision was substantial ($F_{ST} = 0.121$, $R_{ST} = 0.093$), with 44.6% and 55.4% of the among population diversity being attributable to differences between streams ($F_{SR(T)} = 0.054$) and between regions ($F_{RT} = 0.067$), respectively. Analysis of genetic distances and geographic distances did not support a simple model of isolation by distance for these populations. With one exception, neighbor-joining dendrograms from the Cavalli-Sforza and Edwards' chord distances and maximum likelihood algorithms clustered populations by physiogeographic region, although overall bootstrap support was relatively low (53%). Overall, the results given here suggest that coastal cutthroat trout populations are ultimately structured genetically at the level of individual streams. The dynamic balance between gene flow and genetic drift in the subspecies favors a high

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degree of genetic differentiation and population subdivision with the simultaneous maintenance of high heterozygosity levels within local populations. Results are discussed in terms of coastal cutthroat trout ecology along with implications for the designation of evolutionarily significant units pursuant to the U.S. Endangered Species Act of 1973 and analogous conservation units.

INTRODUCTION

A primary focus in conservation genetics is the delineation of intraspecific population structure. Identification of distinct population segments is crucial to the development of comprehensive management plans and the designation of conservation units, such as evolutionarily significant units (ESUs; Waples 1991) under the U.S. Endangered Species Act of 1973 (ESA) (Utter et al. 1993; Milligan et al. 1994). In fisheries, it has been long recognized that species may be composed of many reproductively isolated sub-groups that respond independently to harvest and management activities (Ryman 1983; Moritz 1994 and references therein). This substructuring is perhaps best demonstrated in Pacific salmon and trouts (*Oncorhynchus* spp.) which have a well documented tendency to "home" to their natal streams for spawning (Scheer 1939; Ricker 1972; Quinn 1993). Such phylopatric behavior limits gene flow between populations and promotes the formation of genetically distinct groups that often display both genetic and phenotypic adaptations to local environmental conditions (Taylor 1991). Homing precision is correlated with other life history traits such as duration of freshwater residence and duration and distance of saltwater

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migrations (Quinn 1993) which vary extensively among *Oncorhynchus* species (Groot and Margolis 1991). Consequently, homing rates, and therefore the extent of intraspecific population structure, also vary extensively among these species. Homing is highly developed in some species facilitating the formation of multiple populations within a drainage or even within a single river (e.g., sockeye salmon (*O. nerka*) (Altukhov and Salmenkova 1991; Wood and Foote 1996)), whereas in others homing is less precise and the tendency is to form population segments over large geographic areas (e.g., pink salmon (*O. gorbuscha*) (Aspinwall 1974; Shaklee and Varnavskaya 1994)). Analysis of presumably neutral genetic markers provides one means to determine the basic population structure of a given species, which can then be combined with complementary phenotypic or ecological data to identify adaptively distinct population segments (STOCS 1981; Utter 1981; Utter et al. 1993; Bernatchez 1995).

One salmonid for which conservation genetics has recently become a pressing concern is the coastal cutthroat trout (*O. clarki clarki*). Coastal cutthroat trout have undergone a major range-wide decline over the past two decades (Nehlsen et al. 1991; Trotter et al. 1993), although data are limited and locally some populations remain healthy. The American Fisheries Society Endangered Species Committee concluded that all native naturally spawning populations in the states of California, Oregon and Washington are at some level of risk, being either of special concern or on the threshold of becoming threatened or endangered (Nehlsen et al. 1991); and the United States National Marine Fisheries Service (NMFS) recently listed the coastal cutthroat trout of

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the North Umpqua River in Oregon as endangered pursuant to the ESA (NMFS 1996). Due to the paucity of genetic and ecological information available for coastal cutthroat trout, and the anticipation of future petitioning to list other populations as threatened or endangered, NMFS is currently conducting a coastwide status review for coastal cutthroat trout in an effort to designate ESUs throughout their range (NOAA 1994a).

Coastal cutthroat trout have received little study relative to other more economically important, commercially harvested Pacific salmonids. Although extensive genetic analysis has been performed on inland subspecies of cutthroat (Allendorf and Phelps 1980; Leary et al. 1987; Allendorf and Leary 1988; Forbes and Allendorf 1991), few population level genetic studies have been performed on the coastal subspecies. The major study to date (Campton and Utter 1987) examined allozyme variation in populations from a limited geographic area in Puget Sound, Washington (WA), U.S.A. The authors concluded that coastal cutthroat trout populations were highly structured genetically with geographically adjacent streams in Puget Sound supporting reproductively isolated populations, which were further structured by region. However, broad-scale details about how genetic variation is apportioned in the subspecies remain uncharacterized, as are details on homing precision among native populations, rendering the designation of distinct population segments and appropriate conservation units difficult.

The coastal cutthroat trout is the most widely distributed of 14 currently recognized cutthroat subspecies, exhibiting a wide array of complex life history strategies

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and highly variable migration patterns (Behnke 1992). Its range extends from the Eel River in California, USA, to Gore Point on the Kenai Peninsula in Alaska, USA, with inland penetration generally limited to less than 150 km (Behnke 1992). Important aspects of the migration patterns exhibited by anadromous coastal cutthroat trout are unusual among *Oncorhynchus* species. Anadromous populations are believed to remain near river mouths and estuarine areas and not to migrate to the open ocean or cross large bodies of open water (Johnston and Mercer 1976; Jones 1976) as is common with anadromous populations of other *Oncorhynchus* species (Leggett 1977). In addition, migrations are relatively short, both temporally and spatially, being generally limited to less than six months and less than 100 km (Giger 1972; Johnston 1982; Trotter 1989) whereas migrations of hundreds to thousands of kilometers over periods up to six years or more are common among the other species (Leggett 1977). Previous research has shown (Quinn 1993 and references therein) the duration of saltwater migration to be inversely correlated with homing precision in *Oncorhynchus* species. Therefore, the limited saltwater migrations of coastal cutthroat trout suggest that homing is relatively precise, and consequently, that population subdivision is strong.

Microsatellites are a class of highly polymorphic DNA markers that are ideally suited for studies on the level of intraspecific population structure (Bruford and Wayne 1993). The extensive variability and co-dominant Mendelian inheritance exhibited by microsatellites have stimulated their use in intraspecific population differentiation studies in taxa ranging from mammals (Paetkau and Strobeck 1994; Taylor et al. 1994; Forbes et

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al. 1995) to fishes (Nielsen et al. 1994; Angers et al. 1995; McConnell et al. 1995a; McConnell et al. 1995b; Tessier et al. 1995; Bentzen et al. 1996; O'Reilly et al. 1996; Ruzzante et al. 1996a,b; Scribner et al. 1996; Tessier et al. 1997). Recently, Wenburg et. al. (1996, Chapter 2; see also Olsen et al. 1996, Appendix A) applied microsatellite primers previously developed from a variety of salmonids to genetic analysis in coastal cutthroat trout. These authors developed groups of fluorescently-labeled primer pairs which, after being multiplexed together in a single polymerase chain reaction (PCR), could be visualized in a single lane on an automated DNA sequencer, allowing rapid genetic data acquisition for this subspecies.

Based on our knowledge of coastal cutthroat trout ecology and previous allozyme work by Campton and Utter (1987), it was predicted that coastal cutthroat populations throughout WA would be highly structured genetically, being differentiated at the level of individual streams, which were likely to be further structured by physiogeographic region. Here these predictions are tested by applying the procedures developed by Wenburg et al. (1996, Chapter 2), with minor modifications, to the first broad-scale study of genetic population structuring in coastal cutthroat trout. DNA samples from thirteen native anadromous coastal cutthroat trout populations in WA, and one outgroup population from the inland Yellowstone cutthroat subspecies (*O. clarki bouvieri*), were analyzed using a multiplexed set of six fluorescently-labeled microsatellite primer pairs. Results are discussed in light of coastal cutthroat trout ecology and in relation to other

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Oncorhynchus species with emphasis on implications for the designation of conservation units.

MATERIALS AND METHODS

TISSUE PREPARATION AND DNA EXTRACTION

Tissue samples for the 13 coastal cutthroat trout populations in this study were collected by the Washington Department of Fish and Wildlife (WDFW) as part of a related allozyme study. Six collection areas were designated *a priori* to represent the major physiogeographic coastal regions in WA: Hood Canal (HC), South Puget Sound (SPS), North Puget Sound (NPS), Strait of Juan de Fuca (SJF), North Coast (NC) and South Coast (SC) (Figure 3.1). Except for SJF where three populations were collected, two populations were sampled from within each region (Table 3.1). Juvenile fish were collected by electrofishing within stream reaches ranging in size from approximately 100 m to 2 km, then placed on dry ice in the field before being transferred to -80°C freezers. Samples from the Yellowstone cutthroat subspecies, obtained from a hatchery broodstock (McBride Lake, Montana, USA) that has received periodic inclusion of wild spawners in an effort to maintain heterozygosity levels, were also stored at -80°C. One to six months later, small caudal or pelvic fin clips were removed, placed in 95% EtOH and stored at room temperature. Original sample sizes ranged from 25 to 60 per population (Table 3.1). DNA was isolated from approximately 5 mg of tissue by standard phenol/chloroform DNA extraction methods (Hoelzel and Green 1992) and resuspended

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in 100 µL of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The DNA was then quantified, diluted to 100 ng/µL, stored at -20°C for up to three months, and thawed at room temperature immediately preceding PCRs.

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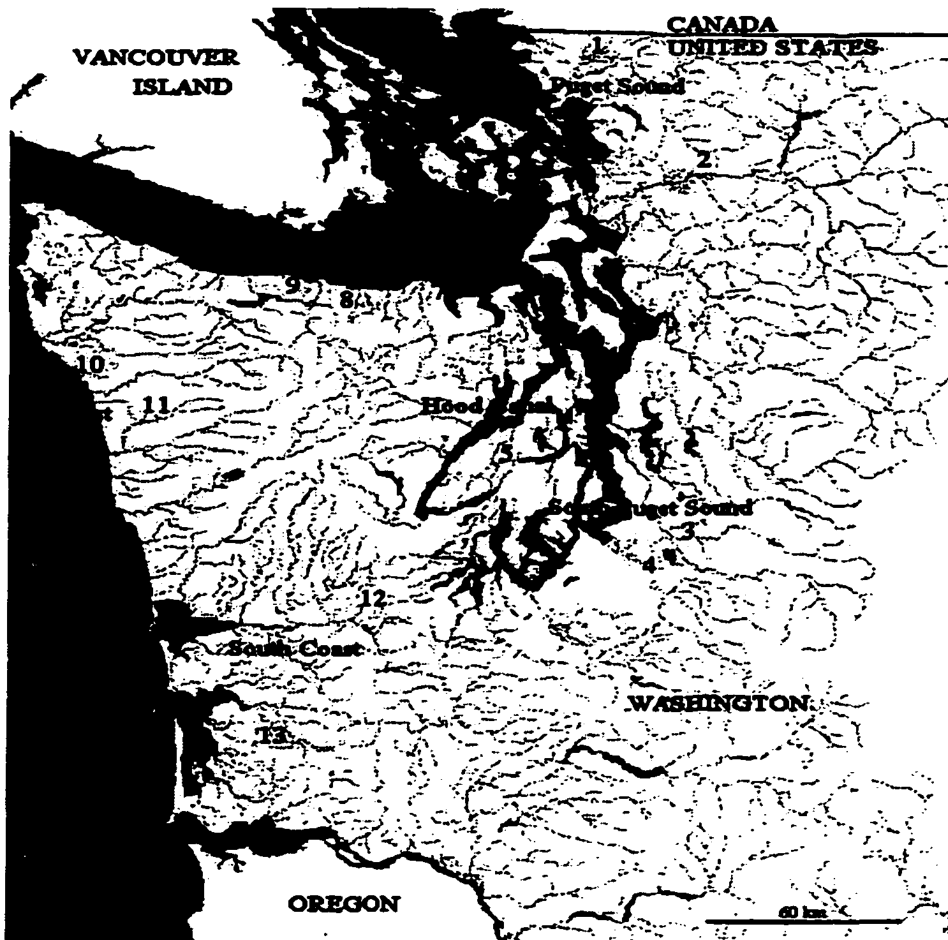

Figure 3.1 Map of sampling locations. North Puget Sound (NPS): (1) Double Ditch Creek, (2) Parker Creek. South Puget Sound (SPS): (3) Covington Creek, (4) Fennel Creek. Hood Canal (HC): (5) Gold Creek, (6) Stavis Creek. Strait of Juan de Fuca (SJF): (7) Gierin Creek, (8) Peabody Creek, (9) Salt Creek. North Coast (NC): (10) Goodman Creek, (11) Snahapish River. South Coast (SC): (12) Wildcat Creek, (13) Oxbow Creek.

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Table 3.1 Sampling locations by region and sample size (*N*). Numbers of samples that exhibited putative steelhead alleles are given in parenthesis.

Region Creek	N
Hood Canal	
Gold Creek	55
Stavis Creek	56(13)
South Puget Sound	
Fennel Creek	43
Covington Creek	50(12)
North Puget Sound	
Parker Creek	50
Double Ditch Creek	50(2)
Strait of Juan de Fuca	
Gierin Creek	60(3)
Peabody Creek	59
Salt Creek	53(10)
North Coast	
Goodman Creek	34(2)
Snahapish River	56
South Coast	
Wildcat Creek	25

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Table 3.1 continued

Oxbow Creek	26
Yellowstone subspecies	
McBride Lake	25
Total	642(42)

MICROSATELLITE AMPLIFICATION AND ALLELE SCORING

PCR conditions, electrophoresis details and methods for scoring amplification products followed those described by Wenburg et al. (1996, Chapter 2). Primer pair sequences and concentrations used in this study correspond to the "Cutthroat A" multiplex set described in that study except for one primer pair (Table 3.2). Oneμ14 was removed due to the strong stuttering tendencies observed in products from this locus in several populations. This primer pair was replaced with Ots4, which, when labeled with the Fam fluorescent label, still allowed for single tube amplification and visualization of all six loci in a single gel lane with all alleles being unambiguously identifiable through size and color separation. Briefly, PCRs were carried out in a Perkin Elmer 9600 thermocycler with a profile consisting of 1 cycle at (94°C for 180 s), 10 cycles at (94°C for 60 s + 56 °C for 30 s + 72°C for 15 s), 14 cycles of (94°C for 30 s + 56 °C for 30 s + 72°C for 15 s), and 1 cycle at (94°C for 30 s + 56 °C for 30 s + 72°C for 300 s). Reactions were carried out in 10 μL volumes comprised of 10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM dNTP's (0.2 mM each), 0.4 units *Taq* polymerase, approximately 100 ng DNA, and primer concentrations as given in Table 3.2. Following

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PCRs, 1 µL product was combined with 3.5 µL formamide, 0.7 µL 50 mM EDTA and 0.25 µL Perkin Elmer GS350 internal size standard, heated to 95°C for 5 m, snap-cooled in ice water and electrophoresed on a 6% denaturing polyacrylamide gel using a Perkin-Elmer Applied Biosystems Inc. 373A automated sequencer.

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Table 3.2 Multiplex set details following "Cutthroat set A" from Wenburg et al. (1996, Chapter 2). Primer concentrations (μM) are given in parentheses for each locus. Size ranges for alleles in loci used for distinguishing between coastal cutthroat trout and steelhead are given in base pairs (b).

Locus (μM)	Primer Sequence F>Forward, R>Reverse	Label[a]	Reference	Source Species	Cutthroat	Steelhead
Omy77 (0.30)	F>5'-CGT-TCT-CTA-CTG-AGT-CAT R>5'-GGG-TCT-TTA-AGG-CTT-CAC-TGC-A	Hex	Morris et al. 1996	*Oncorhynchus mykiss*		
Oneμ11 (0.10)	F>5'-GTT-TGG-ATG-ACT-CAG-ATG-GGA-CT R>5'-TCT-ATC-TTT-CCT-GTC-AAC-TTC-CA	6Fam	Scribner et al. 1996	*Oncorhynchus nerka*		
Ots1 (0.40)	F>5'-GGA-AAG-AGC-AGA-TGT-TGT-T R>5'-TGA-AGC-AGC-AGA-TAA-AGC-A	Tet	David Hedgecock[b]	*Oncorhynchus tshawytscha*	*231-311*	*153-251*
Ots4 (0.08)	F>5'-GAC-CCA-GAG-GAC-AGC-ACA-A R>5'-GGA-GGA-CAC-ATT-TCA-GCA-G	6Fam	David Hedgecock[b]	*Oncorhynchus tshawytscha*		
Sfo8 (0.20)	F>5'-CAA-CGA-GCA-CAG-AAC-AGG R>5'-CTT-CCC-CTG-GAG-AGG-AAA	6Fam	Angers et al. 1995	*Salvelinus fontinalis*	*192-244*	*228-315*
Ssa85 (0.15)	F>5'-AGG-TGG-GTC-CTC-CAA-GCT-AC R>5'-ACC-CGC-TCC-TCA-CTT-AAT-C	Tet	O'Reilly et al. 1996	*Salmo salar*		

Table 3.2 continued

[a] 6Fam=6-carboxyfluorescein (blue), Hex=hexachloro-6-carboxy- fluorescein (yellow), Tet= tetrachloro-6-carboxyfluorescein (green).

[b]University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923, USA.

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Coastal cutthroat trout, steelhead (*O. mykiss*), their F1 hybrids and backcrossed individuals exist within the geographic range covered by this study (Campton and Utter 1985; Hawkins 1997). Further, juveniles of even the two pure species are often difficult to distinguish morphologically (Campton et al. 1991). Therefore, it was necessary to attempt to distinguish between the species in the laboratory. Species-specific allozyme markers have been previously identified for this purpose by Campton and Utter (1985). As part of a related study, the WDFW screened all 13 coastal populations used in this study at three of these loci along with five others they identified as species markers (Bruce Baker, personal communication, WDFW, Olympia, WA, USA). In addition, it was determined that two of the six microsatellite loci used in this study, Ots1 and Sfo8, were effective species markers. Although size ranges did overlap slightly (Table 3.2), the alleles exhibited by these loci for over 90% of the putative "pure" representatives from each species were well within non-overlapping sections of their respective size ranges. As a conservative approach two data sets were created, the first with all steelhead and hybrids identified by both systems (allozyme and microsatellite) removed, and the second with all individuals included; hereafter referred to as data sets #1 and #2, respectively. Except where specifically noted, all results given are from analysis of data set #1. To test the influence these individuals, had they been included, and to estimate the affect that further undetected introgression may have had on the results, various analyses from both data sets were compared.

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DATA ANALYSIS

The following statistical tests were performed using the GENEPOP version 3.1 computer program (Raymond and Rousset 1995; available by anonymous ftp at: isem.isem.univ-montp2.fr in the directory pub/pc/genepop; details of tests and references given here are from GENEPOP documentation where further information can be found) (GENEPOP input file for the analyses in this chapter is given in Appendix B). Tests for deficits from Hardy-Weinberg equilibrium (HWE) were performed for each locus/population combination using an exact test where the *P*-values are estimated without bias using a Markov chain method following the algorithm of Guo and Thompson (1992). Tests for genotypic linkage disequilibrium for all pairs of loci within each population were also made using the Markov chain method. Tests for population differentiation at each locus and over all loci among all populations, and between all possible population pairs, within each region and overall, were made by estimating an unbiased *P*-value of a log-likelihood (*G*) based exact test. As a conservative approach, the *genotypic* differentiation test was invoked in each case. Multilocus estimates of the effective number of migrants (*Nm*) between populations, within regions and overall, were calculated using the private allele method of Slatkin (1985) and were corrected for sample size as given in Barton and Slatkin (1986). For all Markov chain tests, default parameters in GENEPOP for dememorization number (1000), batches (50) and iterations (1000) were invoked. Sequential Bonferroni adjustments were used to judge significance levels for all simultaneous tests in this study (Rice 1989) with an initial α level of 0.05.

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Assessments of genetic subpopulation structuring were made with the aid of the computer programs GENEPOP and FSTAT (Goudet 1995) and manual calculations following equations in Chakraborty and Leimar (1987), Hartl and Clark (1989) and Nei (1987). FSTAT was used to calculate unbiased estimates for analogues of Wright's (1951) *F*-statistics: F_{IS}, F_{IT} and F_{ST} (θ_{IS}, θ_{IT} and θ_{ST}, following Weir and Cockerman 1984). Permutation procedures (N=1000) were used to test whether values were significantly greater than zero by permuting multi-locus genotypes among samples. Heterozygosity components (H_I, H_S, H_R and H_T, where $_I$ = individuals, $_S$ = subpopulations, $_R$ = regions, $_T$ = total) were manually calculated from allelic frequency data to facilitate the partitioning of gene diversity components at the following four hierarchical levels: 1) within populations ($F_{IS} = (H_S - H_I) / H_S$), 2) between populations within regions ($F_{SR(T)} = (H_R - H_S) / H_T$), 3) among regions ($F_{RT} = (H_T - H_R) / H_T$) and 4) overall among populations ($F_{ST} = (H_T - H_S) / H_T$). GENEPOP was also used to calculate ρ_{ST} (Rousset 1996), a weighted analogue of R_{ST} (Slatkin 1995), which is itself an analogue of F_{ST} taking account differences in allele size under a stepwise mutation model of mutation (SMM; Ohta and Kimura 1973) rather than simply identity or non-identity under an infinite alleles model (IAM; Kimura and Crow 1964).

The Mantel test analogue in GENEPOP was used to test for isolation by distance among coastal populations, using F_{ST} (θ)and R_{ST} (ρ)as measures of genetic distance. The geographic distance matrix was constructed by measuring the distances (river + ocean) between populations. Ocean distances were approximated by measuring along coastlines,

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and not necessarily by the most direct route, as this measurement most closely represents the likely migration routes of coastal cutthroat trout.

The following analyses were performed using the PHYLIP software package (version 3.5c; Felsenstein 1993). Nei's (1978) standard distance (D) and the Cavalli-Sforza and Edwards' (1967) chord distance (CSE) were calculated for all populations in GENDIST. The neighbor-joining algorithm in the NEIGHBOR program was used to generate dendrograms from these distance matrixes. The maximum likelihood distance matrixes and dendrograms were calculated in the program CONTML. To assess the robustness of tree topologies, 1000 bootstrap replicates were created in the SEQBOOT program from the original data sets and used as input files for the distance programs before being analyzed for best fit in the CONSENSE program. Dendrogram diagrams were created in DRAWTREE and DRAWGRAM.

RESULTS

MULTIPLEX AMPLIFICATIONS

As a conservative measure, any PCR products that did not exhibit easily scored peaks were considered "unsuccessful" and were excluded from analysis. It is likely that many of these could have been successfully reamplified in single locus reactions. However, due to the relatively small increases in sample sizes that would have been obtained for most loci and the correspondingly small benefits that would have been derived for this study, single locus reamplification was not attempted. Among the 642

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samples from the 14 populations, 42 individuals from six populations exhibited putative "steelhead" alleles (Table 3.1) and were thus excluded from data set #1.

The average number of samples analyzed per population was 43, and the average number of successfully scored products for individual loci across all populations ranged from 33 (Ots1) to 40 (Oneμ11, Omy77 and Ssa85; mean = 38 (88%); Table 3.3). Previous study has shown that multiplex PCRs are often less successful, especially for loci yielding relatively large products, when DNA is extracted from tissue that was initially frozen (Wenburg et al. 1996, Chapter 2). All tissue samples from coastal populations used in this study were frozen before being placed in ethanol, as they were originally collected by WDFW for allozyme study. This treatment appeared to cause a slightly lower success rate for multiplexing in this study as compared to Wenburg et al. (1996, Chapter 2) and other related work in progress (Wenburg, unpublished data).

Table 3.3 Allelic variability at six microsatellite loci in 14 coastal cutthroat trout populations. "A" number of alleles per locus; "R" allelic size range in b; "S" size in b and "F" frequency of the most common alleles; "H_E" expected heterozygosity; "H_O" observed heterozygosity; and "N" number of samples successfully genotyped are given for each population and locus. H_o values representing significant HWE deficits using sequential Bonferroni corrections are marked with * (initial $\alpha = 0.008$ for simultaneous tests across loci) and ** (initial $\alpha = 0.0035$ for simultaneous tests across populations).

Region			Locus			*Mean*
Creek	Omy77	Oneμ11	Ots1	Ots4	Sfo8	Ssa85	*All Loci*

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Table 3.3 continued

Hood Canal								
Gold Creek	*A*	12	3	13	10	12	17	11
	R	119-145	146-148	259-305	101-123	192-240	111-197	-
	S	133	146	275	121	198,212	141	-
	F	0.29	0.80	0.19	0.52	0.22	0.26	0.38
	H_E	0.84	0.35	0.88	0.68	0.85	0.86	-
	H_O	0.89	0.23	0.83	0.32*	0.87	0.84	-
	N	54	52	52	53	52	51	52
Stavis Creek	*A*	16	4	14	7	13	18	12
	R	111-145	144-148	239-295	111-129	192-238	115-197	-
	S	133	146	271	121	198	135	-
	F	0.26	0.56	0.28	0.50	0.24	0.17	0.34
	H_E	0.87	0.60	0.86	0.68	0.86	0.90	-
	H_O	0.86	0.48**	0.79	0.71	0.74	0.86	-
	N	43	42	39	41	43	43	42
South Puget Sound								
Fennel Creek	*A*	12	1	8	5	8	10	7
	R	111-143	146	231-299	111-125	192-216	125-169	-
	S	133	146	239	121	214	153	-
	F	0.24	1.00	0.34	0.69	0.29	0.19	0.46
	H_E	0.86	0.00	0.80	0.47	0.83	0.88	-
	H_O	0.88	0.00	0.55*	0.36	0.95	0.76	-

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Table 3.3 continued

	N	40	42	22	42	19	41	34
Covington Creek	A	13	3	16	8	13	14	11
	R	111-155	146-148	201-301	111-131	194-226	115-179	-
	S	129	146	277	121	210	139	-
	F	0.21	0.59	0.41	0.65	0.22	0.20	0.38
	H_E	0.90	0.58	0.80	0.55	0.89	0.90	-
	H_O	0.67*	0.51	0.69	0.43*	0.83	0.85	-
	N	36	35	35	37	36	33	35
North Puget Sound								
Parker Creek	A	11	3	11	4	10	7	8
	R	107-143	146-148	243-295	111-123	194-242	129-149	-
	S	119	147	283	121	214	143	-
	F	0.383	0.75	0.38	0.61	0.31	0.51	0.49
	H_E	0.77	0.42	0.83	0.53	0.84	0.66	-
	H_O	0.87	0.28*	0.63	0.72	0.81	0.83	-
	N	47	47	16	47	26	47	38
Double Ditch Creek	A	11	4	8	5	14	15	10
	R	109-141	144-148	263-295	109-127	194-240	109-175	-
	S	133	146	263	121	212	141	-
	F	0.33	0.62	0.38	0.48	0.20	0.27	0.38
	H_E	0.81	0.54	0.79	0.61	0.89	0.85	-

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Table 3.3 continued

	H_O	0.89	0.58	0.81	0.60	0.80	0.85	-
	N	47	48	21	48	30	47	40
Strait of Juan de Fuca								
Gierin Creek	A	11	3	13	8	10	8	9
	R	109-141	144-148	239-293	101-123	192-228	125-161	-
	S	129	148	265,277	121	214	145	-
	F	0.25	0.54	0.18	0.22	0.28	0.23	0.28
	H_E	0.84	0.56	0.90	0.85	0.85	0.83	-
	H_O	0.80**	0.63	0.91	0.72*	0.86	0.82	-
	N	55	57	57	43	57	56	54
Peabody Creek	A	6	3	7	3	8	5	5
	R	111-141	144-148	261-277	111-121	198-222	135-151	-
	S	135	146	271	111	200	135	-
	F	0.51	0.76	0.44	0.75	0.29	0.59	0.56
	H_E	0.68	0.38	0.71	0.41	0.81	0.56	-
	H_O	0.66	0.38	0.58**	0.44	0.90	0.53	-
	N	53	56	50	54	52	51	53
Salt Creek	A	13	3	12	7	12	13	10
	R	109-143	146-148	239-301	109-127	192-242	103-179	-
	S	129	146	277	121	210	139	-
	F	0.18	0.63	0.44	0.52	0.22	0.26	0.38

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Table 3.3 continued

	H_E	0.90	0.54	0.77	0.65	0.88	0.87	-
	H_O	0.73**	0.50	0.65	0.53	0.86	0.81	-
	N	41	38	26	34	43	37	37
North Coast								
Goodman Creek	A	10	4	12	6	13	17	10
	R	109-155	144-148	231-301	111-127	192-244	115-201	-
	S	111	146	301	111	214	147	-
	F	0.22	0.69	0.26	0.42	0.30	0.19	0.35
	H_E	0.85	0.49	0.88	0.71	0.88	0.91	-
	H_O	0.59*	0.42	0.86*	0.77	0.87	0.78	-
	N	29	31	29	30	30	32	30
Snahapish River	A	8	4	11	5	9	16	9
	R	109-141	144-148	235-311	111-125	200-244	119-205	-
	S	137	146	255	111	214	147	-
	F	0.26	0.44	0.22	0.42	0.32	0.23	0.31
	H_E	0.84	0.66	0.85	0.69	0.82	0.89	-
	H_O	0.47*	0.49	0.80*	0.76	0.76*	0.92	-
	N	51	55	49	51	49	53	51
South Coast								
Wildcat Creek	A	8	1	10	5	10	12	8
	R	121-155	146	243-289	111-127	192-238	119-203	-
	S	141	146	269,281	121	210	137	-

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Table 3.3 continued

	F	0.43	1.00	0.23	0.36	0.24	0.27	0.42
	H_E	0.75	0.00	0.87	0.76	0.87	0.86	-
	H_O	0.68	0.00	0.77	0.59	0.87	0.95	-
	N	22	24	22	22	23	22	23
Oxbow Creek	A	12	3	13	5	10	16	10
	R	109-159	146-148	243-297	111-127	192-238	115-197	-
	S	131	146	263	111	214	143	-
	F	0.27	0.81	0.28	0.56	0.35	0.16	0.41
	H_E	0.88	0.33	0.84	0.64	0.79	0.92	-
	H_O	0.96	0.38	0.78	0.71	0.79	0.92*	-
	N	24	24	23	24	24	25	24
Yellowstone								
McBride Lake	A	4	1	5	2	2	3	3
	R	115-137	144	273-295	115-119	206-210	115-119	-
	S	119	144	291	119	210	115	-
	F	0.56	1.00	0.48	0.54	0.54	0.50	0.60
	H_E	0.61	0.00	0.68	0.00	0.51	0.53	-
	H_O	0.50	0.00	0.65	0.00	0.48	0.21*	-
	N	24	23	23	24	23	24	24
Mean All Populations	A	11	3	10	6	10	12	9
	F	0.31	0.73	0.31	0.51	0.28	0.29	0.41
	H_E	0.82	0.40	0..82	0..59	0.83	0.81	0.71

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Table 3.3 continued

	H_O	0.77	0.34	0.73	0.56	0.81	0.78	0.67
	N	40	40	33	39	37	40	38
Total All Populations	*N*	566	574	464	550	507	562	537

VARIABILITY OF MICROSATELLITE LOCI

Considerable variation was observed at the six microsatellite loci studied. Numbers of alleles per locus across all populations ranged from 4 (Oneµ11) to 45 (Ssa85; mean = 24; Table 3.3). Observed heterozygosities averaged over all populations ranged from 34% (Oneµ11) to 81% (Sfo8; mean = 67%; Table 3.3). Allele frequency data for all Washington populations are given in Appendix D, and those for the Yellowstone population are given in Appendix E.

Tests for conformity to HWE indicated a significant deficit of heterozygotes in 11 of 84 (13.1%) cases when analyzing individual loci across populations (initial $\alpha = 0.0035$), and in 16 of 84 (19.0%) cases when analyzing across loci within each population (initial $\alpha = 0.008$). Using data set #2, significant deficits were found in 14 of 84 (16.7%) and 16 of 84 (19.0%) cases, respectively.

Tests for genotypic linkage disequilibrium rejected the null hypothesis of independence in 26 of 210 comparisons (12.4%; $\alpha = 0.0035$) (data not shown). The rejected cases were spread among 14 locus pair combinations with a maximum of 4 out

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of a possible 14 occurring between any pair. For data set #2, rejections increased to 34 of 210 (16.2%) and were similarly distributed.

Population Differentiation and Structure

Log-likelihood (*G*) based exact tests for population differentiation revealed significant differences in genotypic frequencies overall among populations, for each of the six loci independently and combined, and between all pairs of populations within each region ($P < 0.0001$, $P < 0.0001$ and $P \leq 0.017$, respectively; initial $\alpha = 0.008$; Table 3.4). Tests between all possible population pairs revealed significant differences in all but 18 of 1092 pairwise tests (0.016%; initial $\alpha = 0.008$, data not shown). Nine of these 18 non-significant results were at the least variable locus, Oneμ11, and four of them were between the Salt Creek (SJF) and Covington Creek (SPS) populations. Results from data set #2 were similarly significant with the exception of pairwise tests between all possible population pairs where the number of non-significant cases increased slightly to 20 of 1092 (0.018%). Again, the majority of these (13) involved Oneμ11, and five were between the Salt Creek and Covington Creek populations.

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Table 3.4 P-values from G-tests for population differentiation among 13 coastal cutthroat populations at six microsatellite loci (McBride Lake excluded). All values shown were judged significant following sequential Bonferroni adjustments (initial $\alpha = 0.008$).

Comparison	P-value						
	Omy77	Oneμ11	Ots1	Ots4	Sfo8	Ssa85	All loci
All populations	‡[a]	‡	‡	‡	‡	‡	‡
Within regions:							
Hood Canal							
Gold Creek x Stavis Creek	‡	0.002	‡	‡	0.006	‡	‡
South Puget Sound							
Fennel Creek x Covington Creek	‡	‡	‡	0.017	‡	‡	‡
North Puget Sound							
Parker Creek x Double Ditch Creek	‡	‡	‡	‡	‡	‡	‡

Table 3.4 continued

Strait of Juan de Fuca							
Gierin Creek x Peabody Creek	‡	‡	‡	‡	‡	‡	‡
Gierin Creek x Salt Creek	‡	‡	‡	‡	‡	‡	‡
Peabody Creek x Salt Creek	‡	‡	‡	‡	‡	‡	‡
North Coast							
Goodman Creek x Snahapish River	‡	‡	‡	0.009	‡	‡	‡
South Coast							
Wildcat Creek x Oxbow Creek	‡	0.002	‡	‡	‡	‡	‡

[a]Denotes $P < 0.0001$.

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The corrected multilocus estimates for the effective number of migrants between populations per generation (*Nm*) within each region ranged from 0.9 (SJF) to 4.3 (HC; Table 3.5) with an average of 1.7. Excluding HC, *Nm* averaged 1.2 within regions. The overall corrected *Nm* for the 13 populations (McBride Lake excluded) was 3.2; for data set #2, the overall *Nm* increased to 4.7.

Table 3.5 Regional subpopulation structure. F_{SR} estimates for coastal cutthroat trout populations within each region. Values judged to be significantly greater than zero, using permutation procedures described in the text, are marked with an *; "*Nm*" estimated number of effective migrants per generation between populations using the private alleles method as described in the text.

	F_{SR}							
Region	Omy77	Oneμ11	Ots1	Ots4	Sfo8	Ssa85	All Loci	*Nm*
Hood Canal	0.008	0.076*	0.035*	0.014	0.003	0.023*	0.023*	4.3
South Puget Sound	0.031*	.0325*	0.156*	0.020	0.053*	0.043*	0.087*	1.2
North Puget Sound	0.117*	0.412*	0.146*	0.125*	0.059*	0.222*	0.179*	1.3
Strait of Juan de Fuca	0.128*	0.147*	0.112*	0.230*	0.072*	0.192*	0.145*	0.9
North Coast	0.046*	0.125*	0.083*	0.012*	0.032*	0.042*	0.056*	1.3
South Coast	0.150*	0.126*	0.073*	0.090*	0.056*	0.054*	0.087*	1.1

Within population variation accounted for 87.9% of the gene diversity observed (Table 3.5). The remaining 12.1%, the index of gene diversity (F_{ST} = 0.121), was the proportional decrease in total heterozygosity attributable to population subdivision.

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Subdivision at the among streams and among region levels accounted for 5.4% (F_{SR} = 0.054) and 6.7% (F_{RT} = 0.067) of the overall genetic diversity, respectively. Single locus estimates for F_{ST} values ranged from 0.064 (Sfo8) to 0.226 (Oneμ11; Table 3.6) and were all significantly greater than zero. The within region F_{SR} values ranged from 0.023 (HC) to 0.179 (NPS) and were significantly greater than zero in 32 of the 36 single locus estimates (Table 3.7). The overall index of gene diversity decreased slightly for data set #2 (F_{ST} = 0.115).

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Table 3.6 Distribution of genetic diversity among coastal cutthroat trout populations throughout Washington for six microsatellite loci. (McBride Lake excluded).

	Absolute Gene Diversity				Relative Gene Diversity (%)			
	Total (H_T)	Within streams (H_S)	Within individuals (H_I)	Within regions (H_R)	Within streams	Between streams (F_{ST})	Between streams within regions ($F_{SR(T)}$)	Between regions (F_{RT})
$F_{ST}(\theta)$	0.822	0.723	0.681	0.767	87.9	12.1	5.4	6.7
$R_{ST}(\rho)$					91.7	9.3		

Table 3.7 Genetic diversity statistics. "H_T" total gene diversity; "H_S" gene diversity within populations; "D_{ST}" gene diversity due to differences among populations; and F_{ST} (θ) values are given for each locus and over all loci (McBride Lake excluded). All F_{ST} values shown were judged to be significantly greater than zero using permutation procedures described in the text.

Locus	H_T	H_S	D_{ST}	F_{ST}
Omy77	0.906	0.820	0.086	0.095
Oneμ11	0.533	0.413	0.120	0.226
Ots1	0.920	0.815	0.106	0.115
Ots4	0.726	0.625	0.101	0.139
Sfo8	0.894	0.837	0.057	0.064
Ssa85	0.952	0.826	0.126	0.132
All loci	0.822	0.723	0.099	0.121

Similar results were obtained in the R_{ST} analysis with 91.7% of the proportional decrease in heterozygosity attributable to the within population component of gene diversity and 9.3% (R_{ST} = 0.093) attributable to population subdivision. Again for data set #2, the overall index of gene diversity decreased slightly (R_{ST} = 0.089).

Mantel tests for isolation by distance failed to reject the null hypothesis of no correlation between genetic and geographic distance at the α level 0.05, using both measures of genetic distance, F_{ST} and R_{ST} (P = 0.08 and 0.17, respectively).

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Genetic Relationships Among Populations

Bootstrap support for all dendrograms was low on average (53% for CSE and maximum likelihood, 30% for D). However, the CSE and the maximum likelihood estimates yielded trees of identical branch order topology and similar branch lengths, and tended to group populations by physiogeographic region (CSE, Figure 3.2; maximum likelihood not shown). Populations from the NC and SC clustered by region, forming a loose "coastal" group separate from the rest of the populations that were clustered in another large "inland" group. Within this group, the SJF populations clustered together with one SPS population (Covington) that was most closely associated with the Salt Creek population; the two NPS populations formed a cluster and the remaining SPS population (Fennel) and the HC populations formed another. The tree topology generated using Nei's D varied from the other two methods, yielding a tree displaying the same large "coastal" and "inland" groupings, but seemingly random associations of populations within these groups. It should be noted that all trees generated here, as in Figure 3.2, were unrooted, and the relationships are therefore relative. As such, the "inland" populations are not necessarily *derived* from the "coastal" populations, as would be the implication if the tree were rooted.

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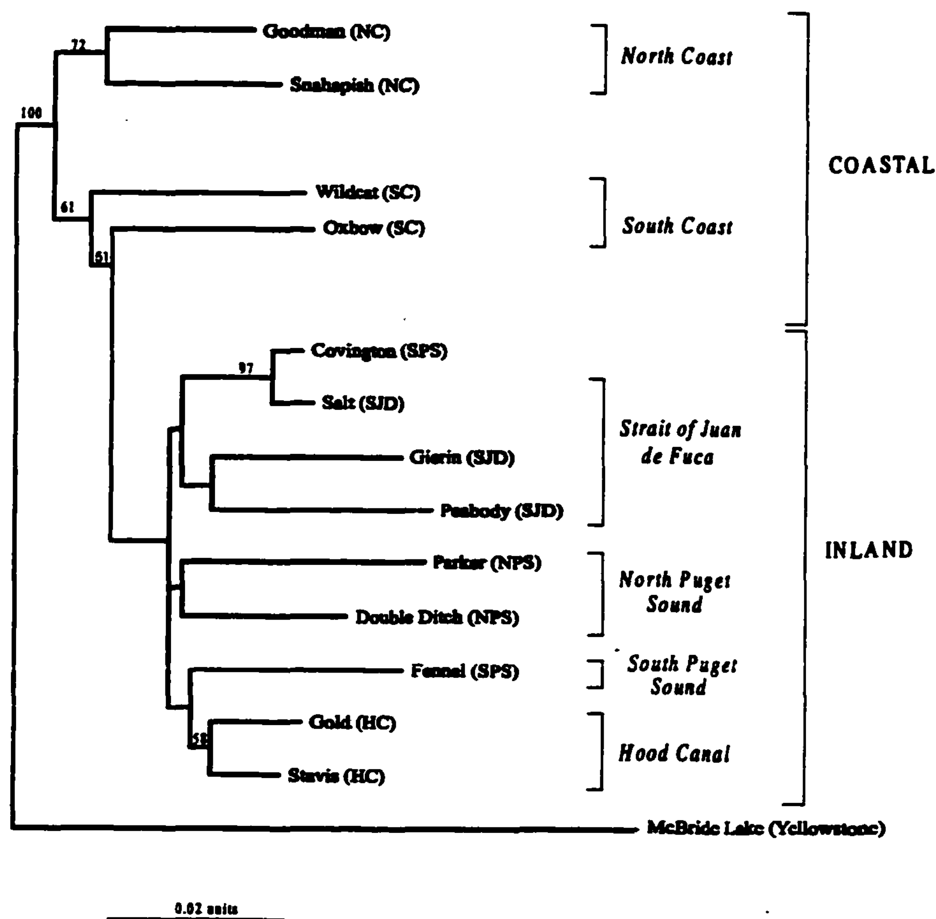

Figure 3.2 Neighbor-joining dendrogram (unrooted) of the genetic relationships among 13 coastal cutthroat trout populations and one Yellowstone cutthroat outgroup population inferred from a matrix of the Cavalli-Sforza and Edwards chord distances for six microsatellite loci. For 1000 bootstrap replicates, node values of 50% and higher are shown.

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Nearly all genetic distance and dendrogram results from PHYLIP were similar when data set #2 was analyzed. Placement of most populations remained identical and bootstrap support decreased only slightly. The single exception was Stavis Creek (HC), which contained the highest number of steelhead/hybrids (13). In data set #2 analysis, Stavis Creek no longer clustered with Gold Creek (HC), but was instead most closely associated with the anomalous Covington Creek (SPS) and Salt Creek (SJF) cluster.

DISCUSSION

These results indicate that all coastal cutthroat trout populations surveyed here are genetically distinct, each comprising distinct breeding units. Simultaneous tests for all populations and all pairwise tests between populations within regions strongly rejected the null hypothesis of no genotypic differentiation, as did all but 18 of 1092 pairwise tests between all possible population combinations. Genetic diversity analysis indicated that a relatively high component of genetic diversity was attributable to differences among populations as opposed to within them, further indicating that creek effects are important contributors to the genetic subdivision in this subspecies (see Table 3.8 for a comparison of F_{ST} values from studies of several salmonid species). The sampling design employed in this study was geared toward a broad-scale survey and as such did not include populations from streams less than 30 km apart. It is therefore possible that at some level, on a finer microgeographic scale, populations would cease to diverge significantly in allelic frequencies at some or even all loci. However, in their study which included adjacent streams, Campton and Utter (1987) found that stream effects were significant in

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7 of 12 intralocus ANOVA comparisons and similarly concluded that populations in the Puget Sound area were structured genetically at this level of subdivision. Populations at the stream level should be regarded as the fundamental component in the genetic structuring of this subspecies, and as the basis for the determination of population segments throughout WA, and likely throughout its full range.

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Table 3.8 Comparisons of genetic diversity analyses for various species.

Species	Geographic Range	Marker Type	# Loci	F_{ST}	# pops	Reference
Oncorhynchus clarki clarki	Washington state	microsatellites	6	0.121	16	this study
O. clarki clarki	Puget Sound, WA	allozymes	31	0.058	21	Campton and Utter 1987
O. mykiss	North America	allozymes	16	0.150	38	Ryman 1983
O. nerka	Cook Inlet, AK	allozymes	26	0.058	13	Ryman 1983
O. gorbuscha	AK to WA	microsatellites	4	0.022	13	Olsen et al. 1998 in press
O. tshawytscha	British Columbia to California	allozymes	25	0.123	86	Utter et al. 1989
Salmo salar	Bay of Fundy to Newfoundland	microsatellites	8	0.054	15	McConnell et al. 1997
S. salar	northern Europe	allozymes	37	0.214	32	Ryman 1983
S. salar	Lake Saint-Jean, Quebec	allozymes	8	0.050[a]	2	Tessier et al. 1995
"	"	microsatellites	5	0.130[a]	"	"
S. salar	Spain and Ireland	microsatellites	3	0.079	7	Sanchez et al. 1996

Table 3.8 continued

"	"	allozymes	6	0.104	"	"
S. trutta	Sweden	allozymes	35	0.367	35	Ryman 1983
Salvelinus fontinalis	La Mauricie national park, Canada	microsatellites	5	0.370	26	Angers and Bernatchez 1998
Gadus morhua	Northwest Atlantic shelf	microsatellites	6	0.015	6	Bentzen et al. 1996

[a]Average of single locus values reported.

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Comparison of F_{ST} and R_{ST} analyses can yield insight into the forces shaping population subdivision. Traditional F-statistic analysis of gene diversity under an IAM assumes each mutation is to a completely new allelic state, erasing any memory of the prior state. Genetic similarity between populations is therefore attributable to migration or recent divergence from a common ancestor. However, the mutational processes of microsatellites do not erase all information about the ancestral allelic state, as mutations tend to at least roughly proceed following a SMM (but see also Di Rienzo et al. 1994 and Angers and Bernatchez 1997 and references therein). When analyzing microsatellites, this apparent memory tends to bias F_{ST} values by overestimating genetic similarity and underestimating coalescence times. Using a SMM for his R_{ST}, Slatkin (1995) showed that it generally provided less biased estimates for demographic parameters than did F_{ST}, given sufficient coalescence times. However, with recently diverged populations, the performance of F_{ST} improves because genetic drift is the dominant process creating local differentiation, and mutational events are of little importance. For the populations examined in this study, R_{ST} was somewhat lower than F_{ST} (0.093 and 0.0121, respectively). This suggests that these populations are of relatively recent evolutionary origin, and that drift and migration have predominated over mutation in shaping the pattern of genetic differentiation.

For resolution of phylogenetic relationships between recently diverged populations, at or below the subspecies level, previous studies have indicated (Takezaki and Nei 1996; Goldstein and Pollock 1997 and references therein) that distance measures

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which do not make inferences about mutational processes (e.g., CSE) will often outperform those based on an specific mutational models such as IAM (e.g., Nei's D) or SMM (e.g., $(\delta\mu)^2$ (Goldstein et al. 1995)). If it is assumed that the populations in this study were structured on a regional scale according to geographic proximity, then the results presented here support this conclusion, as the CSE and maximum likelihood measures did out perform Nei's D in reconstructing population relationships. This also provides further evidence for the predominance of drift as the major evolutionary force in shaping the genetic structure of these populations.

Branch lengths were relatively long and bootstrap support was low for all dendrograms generated in this study, possibly indicating that the regional groupings were somewhat artificial and that the populations within them did not represent disjunct groups. However, the component decrease in heterozygosity attributable to differences among regions was substantial ($F_{RT} = 0.067$), accounting for 55.4% of the overall population subdivision, as opposed to the 44.6% ($F_{SR} = 0.054$) attributable to differences among streams. Nonetheless, to definitively elucidate the extent of regional population structuring in coastal cutthroat trout, additional analyses using larger suites of loci are needed as sampling error and interlocus variance for genetic distances, when using low numbers of microsatellite loci (e.g., < 30), may preclude elucidation of the true phylogenetic relationships among populations (Takezaki and Nei 1996).

In addition, sampling more populations from within smaller geographic regions may reveal that regional structuring of coastal cutthroat trout populations occurs on a

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finer scale than was investigated in this study. Conclusions drawn by Campton and Utter (1987) lend some support to this theory as they found strong clustering of populations (using principal coordinate analysis) for their smaller regional groups from which they sampled multiple adjacent populations (see also Chapter 4).

While bootstrap values for the node separating them were consistently low (30-50%), the large clusters termed "coastal" and "inland" (Figure 3.2) were evident in dendrograms generated from all distance measures. Environmental conditions vary extensively between these regions, with inland streams emptying into areas of protected waters while coastal streams enter directly into the open ocean. Coastal cutthroat trout from these regions exhibit life history variation, smolts migrating from inland streams are of significantly younger age and smaller size than those migrating from coastal streams (Trotter 1989 and references therein). It has been suggested that the physical and environmental characteristics of these two regions have exerted selective pressures that account for the differences in smolt age and size (Johnston 1982). The varying effects of selective pressures, and therefore the potential decrease in fitness for migrants between the regions, may be reflected in the clustering of populations from within these regions, providing an interesting correlation between the genetic infrastructure of this subspecies and various life history attributes.

Analysis of isolation by distance failed to reject the null hypothesis of no correlation between geographic distances and genetic distances, both for F_{ST} and R_{ST}, although the results for the former were nearly significant ($P = 0.08$). This result

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suggests that on the spatial scale of this study, post-glacial population structure of coastal cutthroat trout may have been determined by processes other than dispersal from a single refugium along the contemporary WA coastlines. Separation of the "coastal" and "inland" clusters in the dendrogram may also reflects different patterns of post-glacial recolonization. Perhaps a more complex evolutionary model of migration and divergence from multiple refugia is appropriate for this subspecies, similar to that proposed by Angers and Bernatchez (1998) for brook char populations in Canada.

The *Nm* estimates were very close to 1.0 (0.9-1.3) within five of the six physiogeographic regions in this study (Table 3.7). Although there are several assumptions associated with *Nm* estimates that may not be valid for these populations, they nonetheless are of comparative value. The one-migrant-per-generation (OMPG) is a rule of thumb often used to estimate the amount of gene flow sufficient to minimize the loss of heterozygosity within subpopulations while allowing for divergence of allelic frequencies between them, and has been widely applied by scientists and managers in the context of conservation (Mills and Allendorf 1996). The data presented here lend some empirical support to this theoretical perspective.

Further, this may help to partially reconcile the "cutthroat paradox": namely, in comparison to other *Oncorhynchus* species, coastal cutthroat trout population sizes are often relatively small (tens to hundreds of spawners) and may fluctuate substantially between years (Sumner 1952; Sumner 1962; Jones 1975; NOAA 1994b; Chapter 5) while heterozygosity levels and genetic differentiation are among the highest reported (Utter et

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al. 1980). It can be hypothesized that following the retreat of the Puget glacial lobe approximately 10 thousand years ago (Thorson 1980), considerable gene flow occurred between previously isolated remnants of the subspecies (Behnke 1997; see also Angers and Bernatchez 1998). This may account for the origin of high heterozygosity levels, which are being maintained in the face of the small and fluctuating contemporary local population sizes through persistent gene flow between populations of approximately OMPG, which is nonetheless low enough to permit significant population differentiation between them. Further, coastal cutthroat trout are iteroparous and the genetic implications of overlapping generations also facilitates the maintenance of heterozygosity levels by increasing effective population sizes and reducing the effects of bottlenecks likely to occur in small populations.

Unexpected results in two areas of this analysis require further attention: the higher than expected number of deficits from HWE, and the higher than expected occurrence of genotypic disequilibrium between loci. Although it is possible that the presence of null alleles and/or the mis-scoring of heterozygotes as homozygotes were responsible for these results, several lines of evidence indicate otherwise. First, Wenburg et al. (1996, Chapter 2) found no indication of null alleles when analyzing known cutthroat families at each of these loci. Second, the deviations from HWE observed were spread among the six loci and not heavily concentrated at any one locus, as would be expected for a given locus containing null alleles; similarly, locus pairs exhibiting linkage disequilibrium were spread out with no combination indicating linkage in more than 4 of

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14 comparisons. Third, in a concurrent study by the WDFW using the identical samples for allozyme study, preliminary analysis indicated similar deviations from HWE in roughly the same proportions (Bruce Baker, personal communication, WDFW, Olympia, WA, USA). Finally, previous analysis by Wenburg et al. (1996, Chapter 2) for other populations of coastal cutthroat trout and steelhead did not exhibit such discrepancies when analyzed at the same set of loci.

It can be hypothesized that the methods of collection employed in this study, namely sampling over restricted spatial scales (100 m to 2 km) led to the collection of related individuals, or what Hansen et al. (1997) termed "family sampling". Salmonids are often relatively sedentary during stream residence periods and by collecting samples, especially juveniles, from a limited area over a limited period of time one may tend to capture related individuals (Hansen et al. 1997). Hansen et al. (1997) demonstrated that deviations from HWE could at least be partially attributed to this type of sampling bias. Allendorf and Phelps (1981) also cautioned against the use of juveniles in population studies because of the stochastic affects it may have on allelic frequency distributions; and Campton (1981) found relatively large differences in genotypic frequencies between groups of coastal cutthroat trout captured at different sites within the same stream. It appears likely that the genotypic and Hardy-Weinberg disequilibria found in this study were mainly the result of family sampling error. In addition, sampling of related individuals may have artificially increased the observed differentiation and gene diversity estimates among populations. However, the differentiation between populations here was

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so strong that qualitatively similar results would likely have been obtained regardless of sampling methods. Nonetheless, it cannot be ascertained to what degree this may have affected the gene diversity analyses. Future sampling efforts should endeavor to capture individuals from large spatial and temporal scales to help avoid this sampling bias.

The unexpected association of the Covington Creek (SPS) and Salt Creek (SJF) populations from non-adjacent regions (Figures 3.1 and 3.2) is difficult to explain. These populations were far less differentiated from each other than were any other population pairs, even those from *within* regions. They failed to show significant genotypic differentiation at four of the six loci examined. Only 18 such negative results were found overall in 1092 pairwise tests between all population pairs, with no other population combination accounting for more than one. It is possible that homoplasy, at least in part, may be invoked to explain these results, but it is unlikely that this would affect all four loci in a similar fashion. Past planting of hatchery cutthroat, or even possibly steelhead, from a common broodstock into these areas could also partially explain the association, although there is little data to support such a theory. However, when the putative steelhead and hybrids were included (i.e., in the analysis of data set #2), Stavis Creek (HC), which contained the highest number of steelhead/hybrids (13), moved to cluster with this group. This suggests that relatively high levels of undetected steelhead introgression in Covington and Salt creeks may be partially responsible for their tight association. There is also the possibility that some samples from the two populations were somehow mixed or mislabeled somewhere in the collection and analysis process.

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The discovery of approximately 5% steelhead and hybrids in the populations analyzed here clearly demonstrates the need for further investigation into the issue of natural hybridization between these species, but is well beyond the scope of the present study. Although the various species-marker loci used in this study were not all absolute, and some introgression after several generations of backcrossing using only 10 loci (2 microsatellite and 8 allozyme) may not have been detected, undoubtedly all pure steelhead and F1 hybrids were detected. Comparisons of analyses from the two data sets suggest that, for the purposes of this study, undetected inclusion of some backcrossed individuals would have had little affect. Even with up to 13 individuals in a population exhibiting steelhead alleles, the results obtained with data set #2 were very similar to those from data set #1. As expected, the inclusion of steelhead and hybrids tended to slightly increase the incidence of genotypic linkage and Hardy-Weinberg disequilibrium, and decrease estimates of population subdivision (i.e., F_{ST}), and tests for population differentiation were almost unaffected.

CONCLUSIONS

Based on the high degree of population differentiation and substructuring found here and previous findings by Campton and Utter (1987), it appears that coastal cutthroat trout populations are finely structured into genetically distinct, reproductively isolated units at the level of individual streams, which further appear to be at least loosely structured on a regional scale. Strong differentiation exists among coastal cutthroat trout populations from individual streams with low background levels of reproductive

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migration occurring between them. In this context, high levels of heterozygosity are maintained within populations, even in the face of low local effective population sizes, while allelic frequencies and potential adaptive differences are allowed to accrue between them.

In terms of the designation of ESUs pursuant to the ESA, these results satisfy the first, but not the second of two criteria; as stated by Waples (1991): "A population must satisfy two criteria to be considered an ESU: 1) it must be reproductively isolated from other conspecific population units, and 2) it must represent an important component in the evolutionary legacy of the species". While reproductive isolation is a prerequisite to the formation of locally adapted populations that may "represent important components in the evolutionary legacy of the species", the latter does not necessarily follow from the former. It remains a challenge for interested parties to identify ecologically adaptive differences *or* similarities among the distinct population segments, as identified here, in order to determine the final groupings that represent important components in the evolutionary and adaptive structure of this subspecies (Utter et al. 1993). However, where the data are limited or do not indicate otherwise, the results given here suggest that conservation units should be identified and managed at the level of individual streams.

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CHAPTER 4: MICROGEOGRAPHIC POPULATION STRUCTURE IN THE COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*): GENETIC AND BEHAVIORAL EVIDENCE FOR RESTRICTED GENE FLOW

INTRODUCTION

Details of how genetic variation is apportioned on a microgeographic scale in the coastal cutthroat trout subspecies (*Oncorhynchus clarki clarki*) are as yet uncharacterized, as are the associated patterns of genetic exchange and isolation among populations. The designation of distinct population segments and appropriate conservation units is therefore difficult. The need to resolve these issues in order to develop viable assessment and management strategies is exemplified by the rangewide decline of the coastal cutthroat trout and its recent listing as endangered under the U.S. Endangered Species Act of 1973 (NMFS 1996). Previous allozyme analysis by Campton and Utter (1987) on coastal cutthroat trout in Puget Sound, WA, indicated that distinct breeding units may be structured at the tributary creek level. Wenburg et al. (1998, Chapter 3) supported this conclusion, albeit on a larger scale, as they found 13 populations from throughout Washington to be highly differentiated using microsatellite analysis. However, the study design in Wenburg et al. (1998, Chapter 3) did not include populations that were less than 30 km apart and the authors detailed the need for more microgeographic study. Similarly, Campton and Utter (1987) focused mainly on the partitioning of variance components among drainages and regions in Puget Sound and not

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on differences among streams. Whether or not adjacent creeks and tributaries constitute genetically distinct populations remains uncertain.

The formation of subpopulation structure in any species is predicated on the existence of distinct breeding units that can only be formed and maintained through restriction of gene flow between them. Gene flow between populations may be limited by pre- and postzygotic reproductive isolating barriers operating on various temporal and spatial scales (Avise 1994). As gene flow between groups is reduced they may begin to diverge genetically, both for neutral and selective traits. Genetic drift will cause divergence in neutral genetic markers at a rate that is determined by the degree of isolation between populations, their effective population sizes and the mutational properties of the markers themselves. Gene flow can be estimated in a variety of ways, which are divided into two major classes: those involving direct behavioral observation of organism migrational events, and those involving indirect estimates inferred from the spatial distributions of allele frequencies among populations, the most common of which involve various manipulations of Wright's fixation indices or *F*-statistics (1951).

Potential problems exist for both methodologies. Direct observation yields a necessarily restricted view both spatially and temporally, the result being a tendency to miss rare or infrequent migrations. In addition to the potentially serious logistical difficulties, there may also be an inability to determine the genetic effectiveness of a physical straying event. Previous studies with various taxa (Verhulst and van Eck 1996), including salmonids (Quinn et al. 1987; Tallman and Healey 1994), have shown that the

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degree of physical straying estimated from direct observations may suggest much higher levels of gene flow than those inferred genetically. That is, rates of gene flow may be much lower than immigration rates. Possible explanations for this phenomena include strays that do not spawn, assortative mating, reduced spawning success for strays, and reduced fitness for their offspring. Alternatively, with indirect methods, one must make several simplifying assumptions for inferring gene flow estimates from genetic data, which include various population structure models (e.g., infinite island and stepping-stone), equilibrium states (e.g., between genetic drift and mutation), and mutational models (e.g., the infinite alleles model (IAM; Kimura and Crow 1964), and the stepwise mutation model (SMM; Ohta and Kimura 1973)). A methodology involving both approaches may help to circumvent some of the hazards of each and potentially provide multiple independent measures of gene flow and associated population parameters.

In this study, genetic analysis at 10 microsatellite loci for 10 populations of coastal cutthroat trout from Hood Canal, WA, and behavioral analysis from physical tracking of tagged individuals between four adjacent creeks were combined in order to test the following hypotheses: 1) individual creeks form the distinct breeding units for this subspecies, and 2) some wild coastal cutthroat trout stray (i.e., physical straying), and further that some of these strays spawn (i.e., reproductive straying) in non-natal creeks. Various indirect estimators of gene flow were also calculated and compared to see if they accurately predict effective numbers of straying individuals between populations as determined through direct observation. In addition, data from Wenburg et al. (1998,

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Chapter 3) for populations from throughout Washington were integrated to provide an overall genetic synthesis, providing the most comprehensive assessment to date of the overall dynamics of coastal cutthroat trout population structure. Finally, the results of this study to are used address several issues concerning the mutational properties of microsatellites and the relative utility of various statistical methods used in their analyses.

MATERIALS AND METHODS

SAMPLE COLLECTION AND DNA PREPARATION

Fin clips were collected from 10 (nine anadromous and one non-anadromous) coastal cutthroat trout populations ($N = 472$) in Hood Canal, Washington, USA (Figure 4.1). Samples were collected from the adjacent creeks of Stavis, Seabeck, Big Beef and Little Anderson, which formed a geographic cluster hereafter referred to as the "central" Hood Canal, from Courtney, Bear and Big Mission creeks, which formed a group hereafter referred to as the "southern" Hood Canal cluster, and from Tarboo and Thorndyke creeks from across Hood Canal to the north. Courtney and Bear creeks are tributaries to the Union River (not sampled) which empties into Hood Canal less than 3 km from the mouth of Big Mission Creek. Samples from one non-anadromous population within Hood Canal, Fulton Creek, were collected from above a 10 m waterfall that has likely presented a barrier to migration for approximately 10-15 thousand years.

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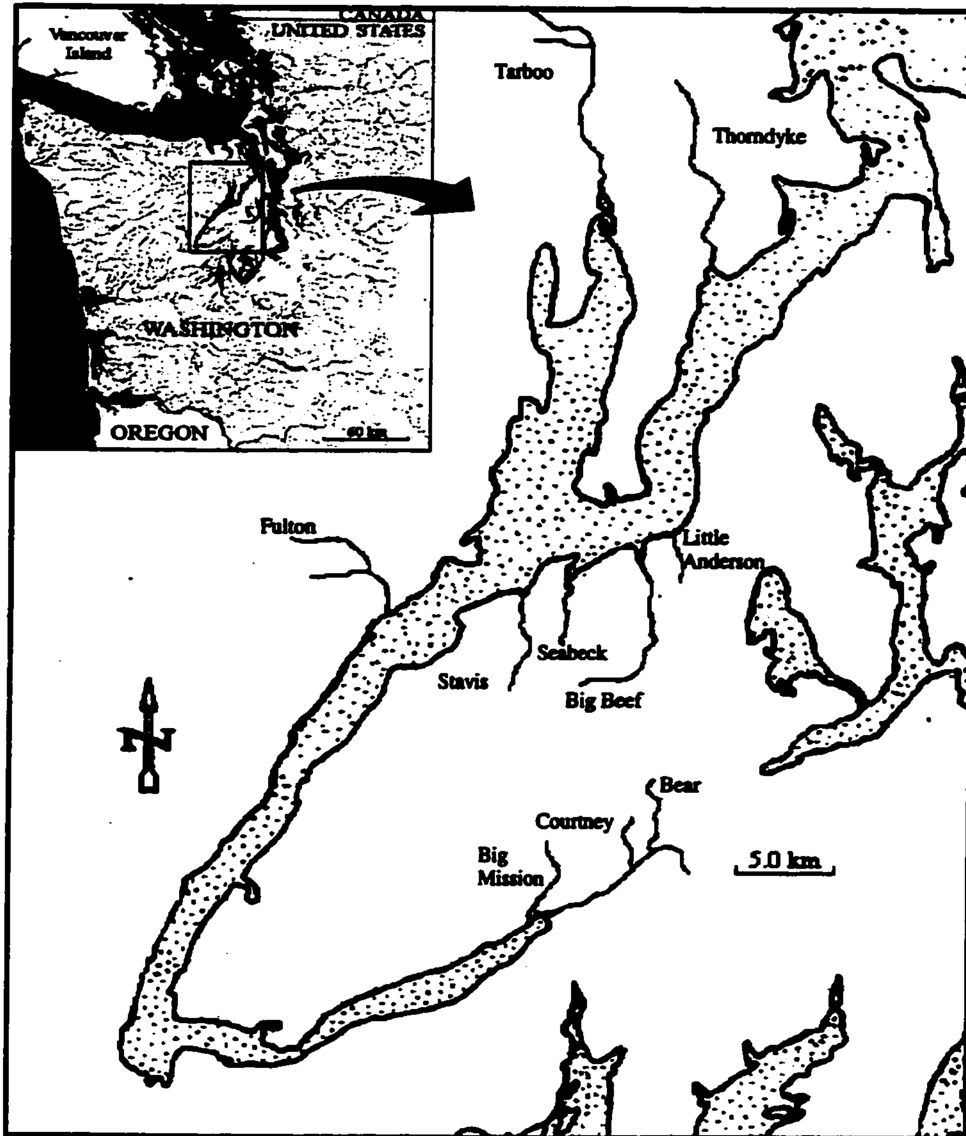

Figure 4.1 Map of sampling locations in Hood Canal, Washington.

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In addition, data from the 14 populations ($N = 642$; 13 anadromous coastal cutthroat populations and one Yellowstone cutthroat subspecies population (*O. clarki bouvieri*)) collected and analyzed at six of the eleven loci used here (set A, details below) by Wenburg et al. (1998, Chapter 3), are periodically included; these will hereafter be referred to as the "statewide" populations in order to distinguish them from the primary populations in this study, which will be referred to as the "Hood Canal" populations. Two of the statewide populations are actually located in Hood Canal, and one of them (Stavis Creek) was included in both data sets. Unless specifically noted, all methodological details given here refer to the Hood Canal samples only. Corresponding details for the statewide populations are given in Wenburg et al. (1998, Chapter 3).

Juveniles (50-130mm) from Fulton, Tarboo and Thorndyke were collected in 1994 from stream reaches of 100, 75 and 300 m, respectively. All other Hood Canal populations were collected by sampling smolting individuals captured in downstream weir traps during the spring outmigration period (March – May) of 1994. Individuals were randomly selected from throughout this period and without regard for size (except Stavis, see below) to avoid the inclusion of related individuals and to preclude the sampling of single age classes (scale analysis indicated that outmigrating smolt from these creeks range in age from one to four years, Chapter 5). Small caudal fin clips (approx. 0.5cm^2) were placed immediately in vials containing 95% EtOH and stored at ambient temperature for 1 day to 1 year. DNA was isolated from tissue using a quick

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lysis protocol (details as in Olsen et al. 1996, Appendix A), resuspended in 100 µL of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), stored at -20°C, and thawed at room temperature immediately preceding polymerase chain reactions (PCRs).

MICROSATELLITE ANALYSIS

Samples from the 10 Hood Canal populations described above were genotyped using two multiplexed sets of fluorescently-labeled microsatellite primer pairs, compromising a total of eleven loci (Table 4.1). PCR conditions, electrophoresis details and methods for scoring amplification products followed those described by Wenburg et al. (1996, Chapter 2; 1998, Chapter 3) and Olsen et al. (1996, Appendix A). Primer pair sequences and concentrations for the six-locus cutthroat set A in this study are identical to the one used by Wenburg et al. (1998, Chapter 3), which is itself slightly modified from set A in Wenburg et al. (1996, Chapter 2). The five-locus set C represents a novel multiplex set developed for this study. Techniques for development of this multiplex set follow those given by Wenburg et al. (1996, Chapter 2) and Olsen et al. (1996, Appendix A).

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Table 4.1. Primer and multiplex set details.

Locus (µM)	Primer Sequence F>Forward, R>Reverse	Label[a]	Reference	Source Species
Set A	Annealing Temperature = 56°C			
Omy77	F>5'-CgT-TCT-CTA-CTg-AgT-CAT	Hex	Morris et al.	*Oncorhynchus*
(0.30)	R>5'-ggg-TCT-TTA-Agg-CTT-CAC-TgC-A		1996	*mykiss*
Oneµ11	F>5'-gTT-Tgg-ATg-ACT-CAg-ATg-ggA-CT	Fam	Scribner et al.	*O. nerka*
(0.10)	R>5'-TCT-ATC-TTT-CCT-gTC-AAC-TTC-CA		1996	
Ots1	F>5'-ggA-AAg-AgC-AgA-TgT-TgT-T	Tet	David	*O. tshawytscha*
(0.40)	R>5'-TgA-AgC-AgC-AgA-TAA-AgC-A		Hedgecock[b]	
Ots4	F>5'-gAC-CCA-gAg-gAC-AgC-ACA-A	Fam	"	"
(0.08)	R>5'-ggA-ggA-CAC-ATT-TCA-gCA-g			
Sfo8	F>5'-CAA-CgA-gCA-CAg-AAC-Agg	Fam	Angers et al.	*Salvelinus*
(0.20)	R>5'-CTT-CCC-CTg-gAg-Agg-AAA		1995	*fontinalis*
Ssa85	F>5'-Agg-Tgg-gTC-CTC-CAA-gCT-AC	Tet	O'Reilly et al.	*Salmo salar*
(0.15)	R>5'-ACC-CgC-TCC-TCA-CTT-AAT-C		1996	
Set C	Annealing Temperature = 52°C			
Oneµ2	F>5'-ggT-gCC-AAg-gTT-CAg-TTT-ATg-TT	Tet	Scribner et al.	*O. nerka*
(0.04)	R>5'-CAg-gAA-TTT-ACA-ggA-CCC-Agg-TT		1996	
Ots100	F>5'-TgA-ACA-TgA-gCT-gTg-TgA-g	Fam	Nelson et al.	*O. tshawytscha*
(0.02)	R>5'-ACg-gAC-gTg-CCA-gTg-Ag		submitted 1998	
Ots101	F>5'-ACg-TCT-gAC-TTC-AA	Hex	Small et al.	"
(0.18)	R>5'-TAT-TAA-TTA-TCC-TC		1998	
Ots103	F>5'-Agg-CTC-Tgg-gTC-CgT-g	Fam	Beacham et al.	"
(0.10)	R>5'-gAC-ATA-gCg-TTC-AgC-ACA-g		1997	
Ssa14	F>5'-CCT-TTT-gAC-AgA-TTT-Agg-ATT-TC	Tet	McConnell	*S. salar*
(0.50)	R>5'-CAA-ACC-AAA-CAT-ACC-TAA-AgC-C		et al. 1995a	

[a]Fam=6-carboxyfluorescein (blue); Hex=hexachloro-6-carboxy- fluorescein (yellow); Tet= tetrachloro-6-carboxyfluorescein (green).
[b]University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923, USA.

PCRs were carried out in a Perkin Elmer 9600 thermocycler with a profile consisting of 1 cycle at (94°C for 180 s), 10 cycles at (94°C for 60 s + X °C for 30 s + 72°C for 15 s), 14 cycles of (94°C for 30 s + X °C for 30 s + 72°C for 15 s), and 1 cycle at (94°C for 30 s + X °C for 30 s + 72°C for 300 s), where X represents the corresponding temperature given in Table 4.1. Reactions were carried out in 10 µL volumes comprised of 10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM

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dNTP's (0.2 mM each), 0.4 units *Taq* polymerase, approximately 100 ng DNA, and primer concentrations as given in Table 4.1. PCR products were electrophoresed on a 6% denaturing polyacrylamide gel using a Perkin-Elmer Applied Biosystems Inc. 373A automated sequencer and scored as given in Wenburg et al. (1996, Chapter 2) and Olsen et al. (1996, Appendix A). It should be noted that numbers of attempted amplifications in the Big Beef sample were not the same for both multiplex sets (see Table 4.3).

Previous work has shown that coastal cutthroat trout and steelhead (*O. mykiss*) hybrids exist within creeks in Hood Canal (Campton and Utter 1985; Hawkins 1997; Wenburg et al. 1998, Chapter 3), and juveniles of even the two pure species are often difficult to distinguish morphologically (Campton et al. 1991; Hawkins 1997), making it necessary to distinguish between the species in the laboratory. Two of the loci used in this study (Sfo8, Ots1) were previously determined by Wenburg et al. (1998, Chapter 3) to be informative in distinguishing between these two closely related species. As a conservative measure individuals exhibiting any putative "steelhead" alleles at either of these loci were removed from analysis.

Tagging and recapture

A permanent weir facility is located at the mouth of Big Beef Creek which, except during major flood events, allows for capture of upstream adult migrants and downstream adult and juvenile migrants. The weir can be maintained with 1 cm gap grates allowing

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capture of adult migrants (> ca. 100 mm) or with fan traps and mesh fencing for capture of adult and juvenile migrants (> ca. 10 mm). The weir can be configured to trap fish moving upstream only and in both directions, or can be opened to allow complete unobstructed movement of fish. From August 5, 1993 to June 12, 1996 various configurations of the weir were maintained in order to track the movements of coastal cutthroat trout into and out of Big Beef Creek. Except for the flood events and occasional maintenance (detailed below), the upstream trap of the weir was maintained and checked on at least a daily basis from August 5, 1993 through June 1, 1994 and from August 15, 1994 through June 12, 1996. During the summer months it was expected that there would be little if any movement of coastal cutthroat trout past the weir. This was confirmed in the summer of 1995 when the weir was maintained with no observed movement of fish. Typically, downstream movement of adults begins in February but smolt outmigration does not begin until March or April (Chapter 5). Due to the increased maintenance involved with the operation of the downstream fan trap and mesh fencing, the normal grates were used except from March/April to June when the movement of smolt was most likely. Specifically, the downstream trap was maintained and checked on at least a daily basis from January 22 through June 1, 1994; February 1 through July 19, 1995, and January 20 through June 12, 1996. Additionally the fans and mesh fencing were in place from April 4 through June 1, 1994 = 61days (d) 1 hour (h); March 3, through July 19, 1995 = 138 d 5 h 44 minutes (m); and March 23, through June 12, 1996 = 78 d 6 h 18 m. Due to flood events and various maintenance requirements, the weir

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was inoperable for approximately 48, 744, and 110 hours during the 1993-1994, 1994-1995 and 1995-1996 seasons, respectively.

Temporary weir traps were constructed on the three creeks immediately adjacent to Big Beef (Seabeck, Stavis, and Little Anderson, 4 and 11 km to the south, and 2 km to the north, respectively; Figure 4.1) and checked on at least a daily basis during the smolt outmigration periods of 1994 and 1995. Specific dates of weir operation follow for each creek, by date and total time in operation with inoperable time following each in parenthesis: Seabeck, March 25-June 16, 1994 = 79 d 14 h 30 m (17 h 30 m) and April 3-June 9, 1995 = 66 d 19 h 10 m (0); Stavis, March 29-June 9, 1994 = 74 d 13 h (34 h) and April 5-June 16, 1995 = 71 d 18 h 20 m (0); Little Anderson, March 25-June 6, 1994 = 73 d 11 h (0) and April 19-June 9, 1995 = 51 d 3 h 30 m (0).

Captured smolt were lightly anesthetized with MS-222 (tricaine: 1 g powder / 20 L water), measured to the nearest mm, weighed to the nearest g (Big Beef Creek only) and had small caudal fin clips removed for DNA analysis along with 5-10 scales from the preferred area for age analysis. In 1994 and 1995, varying percentages (see results for details) of smolt from all 4 creeks were individually marked with visible implant (VI) tags placed between the rays of the anal fin (see Wenburg and George 1995 for details on tagging method and evaluation, Chapter 1). During 1995, for a subsample of smolt, coded wire tags (CWTs; Jefferts et al. 1963) were placed in the tissue at the base of the dorsal fin at Seabeck, Stavis and Little Anderson, and a subsample of smolt from Big Beef had CWTs placed in the snout. These 1.0 x 0.25 mm cylindrical tags are typically

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bar coded and are employed in lethal recapture studies where tags can be extracted from recaptured individuals and read under a microscope. In this study, tags were not extracted, but were merely checked for presence or absence using an electronic hand-held detector. This provided a double marking experiment to test VI tag retention and allowed fish from different creeks to be differentially marked as the detection device was sensitive enough to identify tags placed in various parts of the body. Adults were sampled as detailed above for smolt. In addition, they were checked visually for previous VI tags, checked for CWTs with a detection wand (1996 only), and visually assessed for sex and maturity level.

STATISTICAL ANALYSIS

Tests for deficits from Hardy-Weinberg equilibrium (HWE) were performed for each locus/population combination using an exact test where the *P*-values are estimated without bias using a Markov chain method following the algorithm of Guo and Thompson (1992), as were tests for genotypic linkage disequilibrium for all pairs of loci within each population, using the computer program GENEPOP version 3.1b (GENEPOP input file for these analyses is given in Appendix C). For all Markov chain tests performed in GENEPOP, default parameters for dememorization number (1000), batches (50), and iterations per batch (1000) were invoked. Also in GENEPOP, tests for genotypic differentiation at each locus and over all loci were performed: a) among all populations, and b) between all possible population pairs, by estimating an unbiased *P*-

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value of a log-likelihood (G) based exact test. Sequential Bonferroni adjustments were used for all simultaneous tests in this study (Rice 1989) with an initial α level of 0.05.

For assessment of genetic subpopulation structuring, the computer program FSTAT (Goudet 1995) was used to calculate θ_{ST} and θ_{IS} (following Weir and Cockerman 1984), which are unbiased estimators of Wright's (1951) F_{ST} and F_{IS}, respectively. Permutation procedures (N=1000) were performed in FSTAT to test whether values were significantly greater than zero by permuting multi-locus genotypes among samples for θ_{ST} and alleles within samples for θ_{IS}, and 95% confidence intervals (CIs) for both were calculated by bootstrapping over loci (N=1000). The computer program Rst Calc (Goodman 1997; available at http://helios.bto.ed.ac.uk/evolgen/), was used to calculate ρ_{ST}, which is an unbiased estimator of Slatkin's (1995) R_{ST}, which is itself an analogue of F_{ST} account for the differences in allele size under a SMM rather than simply identity or non-identity under an IAM. Permutation and bootstrap procedures (N=1000) were used to test whether values were significantly greater than zero and to calculate 95% CIs, respectively. Data sets were standardized so alleles were expressed in terms of standard deviations from the global mean before computation of ρ_{ST} statistics. Values reported are those computed from averaging of variance components over loci.

Three methods were used for indirect calculation of gene flow estimates. Multilocus estimates of the effective number of migrants per generation (Nm) between all population pairs and overall in Hood Canal (non-anadromous Fulton Creek population

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excluded), were calculated: 1) using the private allele method of Slatkin (1985) corrected for sample size as given in Barton and Slatkin (1986) in GENEPOP; 2) where $Nm = 1/4\ \theta_{ST} - 1/4$ using FSTAT, with 95% CIs being calculated by substituting values from the upper and lower limits of 95% CIs for θ_{ST} obtained through bootstrap resampling; and 3) where $Nm = 1/4\ \rho_{ST} - 1/4$ using Rst Calc, with 95% CIs being determined directly with bootstrap resampling analysis.

Direct estimates of *Nm* between Big Beef and Stavis, Seabeck and Little Anderson creeks were made from tag recapture data. Using an average generation time of 3 years, and correcting for incomplete tagging and estimated tag loss, ranges of *Nm* were calculated from the three creeks adjacent to Big Beef into Big Beef from 1994 to 1995 and from 1995 to 1996 using:

(1) $$Nm = 3 \times \mathrm{M} \times (\mathrm{P} \times \mathrm{R} \times \mathrm{W})^{-1}$$

where M is the number of tagged migrants captured entering Big Beef from the donor creek, P is the percentage of outmigrating smolt tagged migrating from the donor creek in the previous year, adjusted for time the donor creek weir was inoperable, using:

(2) $$\mathrm{P} = (t/x) \times ((d-i)/d))$$

where t is the number of smolt tagged, x is the number of smolt counted past the weir, i is the number of days the weir was inoperable, and d is the total number of days sampled, and R is the percentage estimated tag retention from double tagging experiments, where

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(3) $R_{CWT} = (rb+r)/rt$

where *rb* and *r* are the number of double-tagged recaptures retaining both tags and the VI tag only, respectively, and *rt* is the total number of double-tagged recaptures, therefore $R_{CWT} = 0.93$, and W is a correction for the percentage of time the weir at Big Beef was inoperable, using:

(4) $W = (d-i)/d$

where *d* is the total number of days sampled at Big Beef, and *i* is the number of days the Big Beef weir was inoperable during that period. During 1994-1995 the weir was maintained for 338 days (8112 hours) including the 744 hours of weir down time, therefore $W_{94-94} = 0.91$, and during 1995-1996 the weir was maintained for 329 days (7896) including 110 hours of weir down time, therefore $W_{95-96} = 0.99$.

Several simplifying assumptions were made for the above calculations. The probability of retention of each tag type was assumed to be independent from the other. There is no direct data to support this assumption, however, it seems intuitively realistic as tag placement techniques and locations varied extensively. Moreover, it was assumed that retention of CWTs in the dorsal fin tissue was equal to that in the snout, as the double tagging experiment included only the latter. To correct for weir down time, it was assumed that migration rates were constant throughout the runs. While this is not entirely true, the limited and sporadic timing of weir down times likely had an averaging out affect on numbers of undetected migrants. In addition, it was assumed that sampling for

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each creek and year spanned the entire period of smolt outmigration. Data from this study (not shown) and several years worth of data collected by the Washington Department of Fish and Wildlife (WDFW) indicate that potential numbers of migrants missed were negligible. It was also assumed that tag retention was independent of fish size. Wenburg and George (1995, Chapter 1) found no correlation of VI tag loss and fish size in coastal cutthroat trout over the range in question here, and several studies have indicated that CWT retention rates are generally greater than 95% and similarly independent of fish size (Blankenship 1990; Blankenship and Tipping 1993; Peterson et al. 1994). Further, it was assumed that tagging procedures did not affect the survival, behavior or homing ability. Blackenship and Tipping (1993) found that return rates for coastal cutthroat trout were similar for VI tagged and untagged fish, Wenburg and George (1995, Chapter 1) found no evidence for increased mortality among smolt or adults associated with VI tagging, and most available evidence suggests that the use of CWTs does not significantly increase mortality or behavior in salmonids (Bergman et al. 1992). However, there is some evidence suggesting that injection of CWTs may cause olfactory tissue damage in chum (O. keta) and pink salmon (O. nerka) fry (< 1 g) and thus may affect homing precision (Morrison and Zajac 1987; Habicht et al. 1998). Due to the much larger size of the smolt tagged in this study, such effects were likely negligible. Finally, for comparisons to the indirect *Nm* estimations, which assume reciprocal gene flow between populations, it was assumed that the number of migrants *into* Big Beef from a given creek were equivalent to the number of strays *from* Big Beef into that creek.

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Isolation by distance (IBD) tests among the nine anadromous Hood Canal populations were performed in GENEPOP, using θ_{ST}, ρ_{ST} and the Cavalli-Sforza and Edwards' (1967) chord distance (CSE; calculated in PHYLIP, see below) as measures of genetic distance. Similarly, IBD tests were performed using number of significantly diverged loci (0-10 from *G*-test results) between populations as a surrogate for genetic distance. GENEPOP performs a test similar to the Mantel test (1967) but uses a rank correlation coefficient and no approximation to provide the distribution of the test statistic under the null hypothesis of independence between the two variables tested (i.e., genetic and geographic distance).

For geographic distances in IBD tests, two matrices were constructed, both with distances (ocean plus creek) approximated between sampling locations from United States Geological Survey (1:24,000 scale) topographic maps. First, ocean distances were approximated by measuring along coastlines, and not necessarily by the most direct route, as it has been reported that coastal cutthroat trout do not migrate across large bodies of open water (Jones 1976; Johnston 1982). However, if cutthroat do migrate across Hood Canal, distances between the Thorndyke and Tarboo and the creeks in the central Hood Canal cluster would have been drastically overestimated (see Figure 4.1). Therefore a second distance matrix was constructed under the assumption that the open water of Hood Canal does not represent a migration barrier by measuring the most direct open water routes between populations (migration across Hood Canal in this region would potentially be < 4 km over depths < 100 m).

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Except for the pairwise $(\delta\mu)^2$ (Goldstein et al. 1995) distances, which were calculated in Rst Calc, the following genetic distance analyses were performed using the PHYLIP software package (version 3.5c; Felsenstein 1993). Pairwise distances of Nei's (1972) standard distance (D) and CSE were calculated in GENDIST. The neighbor-joining algorithm in the NEIGHBOR program was used to generate dendrograms from all distance matrices. The maximum likelihood distance matrices and dendrograms were calculated in the program CONTML. To assess the robustness of the CSE tree topologies, 100 bootstrap replicates were created in the SEQBOOT program from the original data sets and used as input files for distance calculations before being analyzed for best fit in the CONSENSE program. Dendrogram diagrams were created in DRAWTREE and DRAWGRAM.

Using the methods described above, data from Wenburg et al. (1998, Chapter 3) were incorporated for several analyses. Data from the Yellowstone population was combined with that from the nine anadromous Hood Canal populations (Fulton excluded) at eight loci (Ots100 and Ots103 excluded) for calculation of θ_{ST} and ρ_{ST}, and to derive pairwise distances for CSE, D, and $(\delta\mu)^2$. Next, given the highly significant correlation found in this study between geographic distance and CSE (see Results), the 13 statewide populations (Yellowstone excluded) were reanalyzed for IBD using CSE as a measure of genetic distance (only θ_{ST} and ρ_{ST} were used originally). In addition, data from 6 loci (set A) for the statewide and Hood Canal populations were combined in order to derive dendrograms of overall relationships between the 23 combined populations. Finally, the

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data from the Stavis Creek samples from both studies were tested at six loci (set A) for genotypic differentiation (*G*-tests) and for indication of subpopulation structure (θ_{ST}, θ_{IS} and ρ_{ST}). These collections were made independently in alternate years and were comprised primarily of different age classes from the same creek, allowing comparison of the relative amount of year to year variation within populations to that found between them. Based on length at age data collected from scale samples (Chapter 5), only individuals 50-135 mm were used for this comparison to ensure that an overwhelming proportion of each collection represented an age 1 cohort from alternate years.

RESULTS

Within population variability

Genetic variability for the ten coastal cutthroat trout populations analyzed here at ten microsatellite loci is summarized in Table 4.2 (Ots101 excluded, see below). Numbers of total alleles per locus ranged from 3 (Ots103) to 33 (Ssa85), and averaged 3 (Oneμ11 and Ots103) to 15 (Ssa85) across populations, with the total average number of alleles per locus being 17. Expected heterozygosities averaged across populations ranged from 0.16 (Ots103) to 0.87 (Omy77, Ots1 and Ssa85) with an overall average of 0.69. A total of 37 of 172 (21.5%) alleles were private (present in only one population), and were distributed among all ten populations with the exception of Seabeck, with 10 occurring at the most variable locus (Ssa85). When data from Wenburg et al. (1998, Chapter 3) was included, a total of 34 private alleles were observed out of 209 (16.3%). Two loci

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exhibited alleles indicating they were not perfect dinucleotide repeats. Oneμ11 exhibited alleles of 144, 146, 147, 148, and 149 b, indicating a possible point mutation in one of the flanking sequences; Ots103 appeared to amplify a tetranucleotide repeat in coastal cutthroat trout as all three alleles were separated by 4 b (182, 186, and 190 b). Putative steelhead alleles were detected in 24 out of 472 individuals (5%) that were thus excluded from analysis (Table 4.3). Overall success rates for amplification and scoring of multiplex products across all populations and loci was 90.0% (Table 4.3). Allele frequency data for all populations are given in Appendix E, and genotypic data are given in Appendix C.

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Table 4.2. Summary of allelic variability at ten microsatellite loci in ten coastal cutthroat trout populations from Hood Canal, WA. "*A*" number of alleles; "*R*" allelic size range in b; "*S*" and "*F*" size in b and frequency of the most common alleles, respectively; "H_E" expected heterozygosity; "H_o" observed heterozygosity; "*P*" *P*-value of tests for deficits from HWE; "*N*" number of samples successfully genotyped; "*PR*" number of private alleles.

Creek	Stat.	Omy77	Oneμ11	Ots1	Ots4	Sfo8	Ssa85	Oneμ2	Ots100	Ssa14	Ots103	*Mean All Loci*
Thorndyke	*A*	12	4	9	6	11	12	10	11	6	3	8
	R	111-145	146-149	239-305	111-125	192-240	133-199	210-232	176-224	131-149	182-190	-
	S	119	148	271	121	198	141	228	222	139	186	-
	F	0.23	0.54	0.34	0.44	0.26	0.27	0.36	0.25	0.59	0.98	0.43
	H_E	0.87	0.56	0.77	0.64	0.85	0.86	0.82	0.83	0.61	0.04	0.69
	H_O	0.89	0.35	0.95	0.72	0.89	0.96	0.85	0.89	0.67	0.04	0.72
	P	0.555	0.001*	1.000	0.620	0.865	1.000	0.733	0.002*	0.766	1.000	-
	N	47	46	43	47	47	46	46	46	46	48	46
	PR	-	1	-	-	-	-	-	1	-	-	0.2
Tarboo	*A*	15	3	10	4	11	16	12	14	6	3	9
	R	111-147	146-148	239-293	111-127	192-238	125-197	210-238	168-224	131-149	182-190	-
	S	141	146	263	111	198	153	228	222	139	186	-
	F	0.23	0.68	0.26	0.47	0.26	0.34	0.41	0.22	0.66	0.93	0.45
	H_E	0.88	0.49	0.84	0.64	0.84	0.83	0.78	0.89	0.53	0.14	0.69
	H_O	0.94	0.47	0.73	0.63	0.83	0.83	0.65	0.91	0.50	0.11	0.66
	P	0.913	0.623	0.206	0.010	0.331	0.695	0.113	0.658	0.261	0.142	-
	N	33	34	33	35	35	35	34	34	34	35	34
	PR	-	-	-	-	-	-	-	1	-	-	0.1
Fulton	*A*	6	2	11	4	12	13	8	7	4	3	7
	R	113-159	146-148	243-297	109-121	192-226	129-183	200-240	194-248	137-147	182-190	-
	S	113	146	295	121	192	167	206,216	194	139	186	-
	F	0.29	0.77	0.27	0.59	0.27	0.21	0.21	0.42	0.82	0.57	0.44
	H_E	0.80	0.37	0.90	0.59	0.90	0.90	0.88	0.78	0.32	0.59	0.70
	H_O	0.79	0.24	0.69	0.41	0.94	0.82	0.93	0.42	0.35	0.64	0.62

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Table 4.2 continued

	P	0.559	0.177	0.014	0.062	0.324	0.099	0.799	0.001*	1.000	0.745	-
	N	19	17	13	17	17	17	14	12	17	14	16
	PR	-	-	-	-	-	-	2	1	-	-	0.3
Courtney	*A*	11	3	13	4	10	14	11	11	6	2	9
	R	113-137	146-148	239-305	111-123	192-240	125-197	206-232	186-224	135-149	186-190	-
	S	133	146	263,265	121	212	153	228,232	222	139	186	-
	F	0.22	0.77	0.16	0.58	0.37	0.20	0.17	0.50	0.68	0.98	0.46
	H_E	0.88	0.37	0.92	0.56	0.83	0.90	0.90	0.73	0.52	0.04	0.66
	H_O	0.87	0.45	0.91	0.58	0.78	0.96	0.92	0.59	0.56	0.04	0.67
	P	0.015	1.000	0.559	0.058	0.152	0.907	0.594	0.002*	0.840	-	-
	N	23	22	22	24	23	23	26	22	25	26	24
	PR	-	-	-	-	-	-	-	1	-	-	0.1
Bear	*A*	12	3	15	5	12	17	8	12	6	2	9
	R	113-145	146-148	239-305	111-123	192-228	111-197	212-232	176-232	135-147	186-190	-
	S	131	146	271	111	212	153	228	222	139	186	-
	F	0.20	0.66	0.21	0.61	0.38	0.29	0.26	0.30	0.75	0.96	0.46
	H_E	0.88	0.52	0.90	0.58	0.81	0.89	0.85	0.85	0.43	0.08	0.68
	H_O	0.85	0.59	0.90	0.63	0.83	0.90	0.86	0.87	0.46	0.08	0.70
	P	0.107	0.919	0.649	0.771	0.872	0.708	0.474	0.118	0.826	1.000	-
	N	27	29	29	27	29	29	21	23	24	26	26
	PR	-	-	-	-	-	-	-	1	-	-	0.1
Big Mission	*A*	12	3	12	8	11	17	10	8	6	2	9
	R	113-145	146-148	239-293	109-125	194-238	115-189	214-238	172-226	131-147	182-190	-
	S	133	146	271	121	198	153	228	192	139	186	-
	F	0.24	0.66	0.27	0.47	0.30	0.24	0.28	0.24	0.80	0.93	0.44
	H_E	0.86	0.52	0.86	0.72	0.82	0.89	0.83	0.85	0.36	0.14	0.69
	H_O	0.85	0.47	0.77	0.45	0.89	0.85	0.81	0.63	0.40	0.15	0.63
	P	0.530	0.124	0.005*	0.000*	0.938	0.140	0.390	0.013	1.000	1.000	-
	N	40	38	35	38	37	40	27	19	30	27	33
	PR	-	-	-	-	1	-	-	-	-	-	0.1

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Table 4.2 continued

Stavis	*A*	13	4	13	4	12	17	13	12	4	3	10
	R	113-145	144-148	239-295	111-123	192-238	125-197	210-234	176-224	131-147	182-190	-
	S	127	146	271	121	198	141	228	222	139	186	-
	F	0.19	0.63	0.22	0.49	0.29	0.19	0.21	0.31	0.67	0.89	0.41
	H_E	0.90	0.55	0.88	0.65	0.83	0.91	0.89	0.84	0.51	0.20	0.72
	H_O	0.91	0.53	0.85	0.61	0.85	0.91	0.89	0.82	0.53	0.17	0.71
	P	0.721	0.072	0.103	0.232	0.156	0.544	0.665	0.154	0.919	0.210	-
	N	45	47	47	46	47	44	47	45	47	47	46
	PR	-	-	-	-	-	-	-	1	-	-	0.1
Seabeck	*A*	12	3	10	6	9	13	9	14	4	2	8
	R	113-141	146-148	239-295	109-123	194-238	125-163	214-232	172-226	131-147	186-190	-
	S	123	146	271	121	212	153	228	224	139	186	-
	F	0.26	0.49	0.18	0.61	0.26	0.270	0.29	0.28	0.65	0.93	0.42
	H_E	0.87	0.63	0.85	0.55	0.82	0.87	0.83	0.84	0.53	0.12	0.69
	H_O	0.85	0.47	0.92	0.49	0.82	0.77	0.88	0.67	0.58	0.13	0.66
	P	0.443	0.049	0.890	0.005*	0.508	0.116	0.862	0.015	0.830	1.000	-
	N	39	38	36	37	39	39	40	39	40	38	39
	PR	-	-	-	-	-	-	-	-	-	-	0
Big Beef	*A*	14	4	17	8	15	17	12	14	9	3	11
	R	111-145	144-148	239-305	111-133	192-240	109-197	210-234	172-226	131-149	182-190	-
	S	133	146	271	111	198	153	228	222,224	139	186	-
	F	0.26	0.55	0.23	0.39	0.29	0.37	0.37	0.17	0.55	0.91	0.41
	H_E	0.87	0.61	0.87	0.67	0.86	0.82	0.82	0.90	0.67	0.16	0.73
	H_O	0.85	0.40	0.74	0.66	0.82	0.79	0.83	0.86	0.47	0.17	0.66
	P	0.486	0.000*	0.000*	0.538	0.055	0.189	0.066	0.075	0.000*	1.000	-
	N	92	93	72	93	92	94	47$^{\oplus}$	44$^{\oplus}$	43$^{\oplus}$	46$^{\oplus}$	72
	PR	-	-	-	1	-	-	-	-	-	-	0.1
Little	*A*	12	3	12	4	10	14	10	12	4	3	8

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Table 4.2 continued

Anderson	*R*	111-139	146-148	239-305	111-123	192-242	105-195	210-232	172-226	131-147	182-190	-
	S	137	146	271	121	198	153	228	222	139	186	-
	F	0.19	0.66	0.23	0.43	0.30	0.31	0.30	0.37	0.82	0.94	0.46
	H_E	0.87	0.51	0.88	0.67	0.81	0.85	0.84	0.82	0.32	0.11	0.67
	H_O	0.87	0.45	0.87	0.62	0.73	0.81	0.88	0.69	0.27	0.12	0.63
	P	0.398	0.096	0.584	0.354	0.086	0.000*	0.717	0.018	0.005*	1.000	-
	N	52	53	53	53	52	53	51	49	52	52	52
	PR	-	-	-	-	-	1	-	-	-	-	0.1
Mean All Creeks	*A*	12	3	12	5	11	15	10	12	6	3	9
	F	0.23	0.64	0.24	0.51	0.30	0.27	0.29	0.31	0.70	0.90	0.44
	H_E	0.87	0.51	0.87	0.63	0.84	0.87	0.84	0.83	0.48	0.16	0.69
	H_O	0.87	0.44	0.83	0.58	0.84	0.86	0.85	0.74	0.48	0.17	0.67
	PR	-	0.1	-	0.1	0.1	0.1	0.2	0.6	-	-	-
	N	42	42	38	42	42	42	35$^{\Phi}$	33$^{\Phi}$	36$^{\Phi}$	36$^{\Phi}$	39
Total All Creeks	*A*	19	5	25	12	23	33	18	25	9	3	17
	R	107-159	144-149	231-311	101-133	192-244	103-205	200-254	168-248	131-149	182-190	-
	N	417	417	383	417	418	420	353$^{\Phi}$	333$^{\Phi}$	358$^{\Phi}$	359$^{\Phi}$	388
	PR	-	1	-	1	1	1	2	6	-	-	1.2

* Indicates significant deficits from HWE using sequential Bonferroni corrections for 10 simultaneous tests across loci (initial $\alpha = 0.05/10 = 0.005$).

$^{\Phi}$Amplification was attempted on only 50 samples for Big Beef at these loci, in contrast to the 99 samples amplified at the other six loci (see TableN).

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Table 4.3 Prevalence of putative steelhead alleles and overall microsatellite amplification success. "S" numbers of individuals identified with putative steelhead alleles and removed from each population; "N" sample size after putative steelhead removed, and "Mean %" mean percentage of samples successfully amplified and scored, after steelhead were removed are given for each population.

Creek	S	*N*	Mean %
Thorndyke	0	50	0.92
Tarboo	1	35	0.97
Fulton	0	19	0.84
Courtney	3	26	0.92
Bear	4	29	0.90
Big Mission	3	47	0.70
Stavis	2	48	0.96
Seabeck	10	40	0.98
Big Beef	1	99/50*	0.90
Little Anderson	0	55	0.95
Total:	24	448	0.90

*For multiplex set A, $N = 99$, and for multiplex set C, $N = 50$.

Tests for conformity to Hardy-Weinberg equilibrium (HWE) indicated a significant deficit of heterozygotes in all 10 populations for Ots101 (initial $\alpha = 0.005$, data not shown), likely indicating the presence of significant numbers of null alleles. Therefore, this locus was excluded from analysis. Among the other 10 loci, deficits were found in 12 of 100 comparisons which were spread out across six loci and seven populations, with no more than three occurring at a single locus or within a given population (Table 4.2).

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Tests for genotypic linkage disequilibrium rejected the null hypothesis of independence in only 25 out of 450 (5.5%) pairwise comparisons that were spread among 19 different locus combinations, with a maximum of three occurring between any pair (initial $\alpha = 0.005$; data not shown). This is just slightly above the number of rejections expected by chance alone at this α level (0.05), indicating that the loci used here did not exhibit significant linkage.

For the two Stavis collections, tested as separate populations (data not shown), values for θ_{ST} and ρ_{ST} were not significant and 95% CIs for both overlapped zero. Further, θ_{IS} values were not significantly greater than zero in each collection independently or when pooled into one population, and again in both cases 95% CIs overlapped zero. Tests for genotypic differentiation between the two Stavis collections revealed evidence for panmixia at 5 of 6 loci, with the exception being Ots4. Overall, the year to year variation within creeks was negligible when compared to that between creeks. Therefore, fish from all age classes, within each creek, were pooled for analysis.

Among Population Variability

Log-likelihood (*G*) based exact tests for population differentiation among all populations revealed significant differences at all loci ($P<0.0001$; Table 4.4). Similarly, all 45 pairwise comparisons revealed significant differences between populations over all loci combined. Results from pairwise comparisons at individual loci revealed a more complex picture of differentiation, with significant genotypic differences in 304 of 450

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pairwise tests (0.68%; $P<0.05$). Populations were significantly differentiated at as many as ten loci (Thorndyke and Fulton) and as few as one locus (Courtney and Bear) indicating various levels of differentiation between populations. IBD indicated that the number of differentiated loci between populations was positively correlated with the geographic distance between them (Fulton excluded; $P = 0.005$). In 40 of 45 pairwise comparisons, populations were differentiated at five or more of the 10 loci. Only four comparisons differed at three or fewer loci, all occurring within the cluster of southern Hood Canal creeks (Big Mission, Courtney and Bear). As expected, the non-anadromous population, Fulton Creek, was the most highly differentiated, exhibiting heterogeneity from all other populations at a minimum of seven loci.

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Table 4.4. Probability values from *G*-tests for genotypic differentiation at 10 microsatellite loci among 10 coastal cutthroat populations in Hood Canal, WA. Results from tests at each locus independently and across all 10 loci are given overall among populations with and without Fulton Creek, and for pairwise comparisons between all population pairs. "No.*" numbers of loci at which genotypic homogeneity was rejected are given for each pairwise comparison (right hand column) and for each locus across all pairwise comparisons (bottom row).

	P – value											
Comparison	Omy77	Oneμ11	Ots1	Ots4	Sfo8	Ssa85	Oneμ2	Ots100	Ssa14	Ots103	*All loci*	No.*
Overall:												
Fulton included	‡*	‡*	‡*	‡*	‡*	‡*	‡*	‡*	‡*	‡*	‡*	10
Fulton excluded	‡*	‡*	‡*	‡*	‡*	‡*	‡*	‡*	‡*	0.024*	‡*	10
Pairwise:												
Thorndyke vs. Tarboo	‡*	0.001*	‡*	0.125	0.009*	‡*	0.200	‡*	0.025	0.402	‡*	6
Thorndyke vs. Fulton	‡*	0.006*	‡*	0.039*	‡*	‡*	‡*	‡*	0.006*	‡*	‡*	10
Thorndyke vs. Courtney	‡*	0.001*	‡*	0.329	‡*	‡*	0.001*	‡*	‡*	1.000	‡*	8
Thorndyke vs Bear	‡*	‡*	‡*	0.011*	‡*	‡*	0.090	0.001*	‡*	0.704	‡*	8
Thorndyke vs. Big Mission	‡*	‡*	‡*	0.063	‡*	‡*	0.010*	0.010*	‡*	0.156	‡*	8
Thorndyke vs. Stavis	‡*	‡*	‡*	0.032	‡*	0.001	0.008	‡*	0.004	0.087	‡*	8
Thorndyke vs. Seabeck	‡*	0.006*	‡*	0.072	0.006*	‡*	0.032	‡*	0.020	0.080	‡*	6
Thorndyke vs. Big Beef	‡*	‡*	0.016	0.307	‡*	‡*	0.260	‡*	0.078	0.132	‡*	5
Thorndyke vs. Little Anderson	‡*	‡*	‡*	0.153	‡*	‡*	0.003*	‡*	0.001*	0.514	‡*	8
Tarboo vs. Fulton	‡*	0.055	‡*	‡*	‡*	‡*	‡*	‡*	0.533	‡*	‡*	8
Tarboo vs. Courtney	0.001*	0.151	‡*	0.005*	0.002*	‡*	0.009*	0.001*	0.002*	0.193	‡*	8
Tarboo vs. Bear	0.311	0.960	‡*	0.047	0.188	0.002*	0.065	0.005*	0.275	0.148	‡*	3

Table 4.4 continued

Tarboo vs. Big Mission	‡*	0.835	‡*	0.001*	0.003*	0.004*	0.147	‡*	0.006*	1.000	‡*	7
Tarboo vs. Stavis	‡*	0.205	‡*	0.006*	0.009*	‡*	0.007*	‡*	0.224	0.349	‡*	7
Tarboo vs. Seabeck	‡*	0.089	0.002*	‡*	0.501	‡*	‡*	‡*	0.292	0.032	‡*	6
Tarboo vs. Big Beef	‡*	0.077	‡*	0.257	0.016	‡*	0.055	‡*	0.054	1.000	‡*	4
Tarboo vs. Little Anderson	‡*	0.749	‡*	0.080	0.034	0.002*	‡*	0.002*	0.106	1.000	‡*	5
Fulton vs. Courtney	‡*	1.000	‡*	0.666	‡*	‡*	‡*	‡*	0.038	‡*	‡*	7
Fulton vs. Bear	‡*	0.029	‡*	‡*	‡*	‡*	‡*	‡*	0.984	‡*	‡*	8
Fulton vs. Big Mission	‡*	0.031*	‡*	0.072	‡*	‡*	‡*	‡*	0.213	‡*	‡*	8
Fulton vs. Stavis	‡*	0.062	‡*	0.001*	‡*	‡*	‡*	‡*	0.022*	0.001*	‡*	9
Fulton vs. Seabeck	‡*	0.005*	‡*	0.796	‡*	‡*	‡*	‡*	0.015*	‡*	‡*	9
Fulton vs. Big Beef	‡*	0.014*	‡*	0.001*	‡*	‡*	‡*	‡*	0.076	‡*	‡*	9
Fulton vs. Little Anderson	‡*	0.017*	‡*	0.001*	‡*	‡*	‡*	‡*	0.240	‡*	‡*	9
Courtney vs. Bear	0.146	0.082	0.163	0.001*	0.289	0.056	0.497	0.049	0.103	1.000	0.0003*	1
Courtney vs. Big Mission	0.343	0.070	0.001*	0.043	‡*	0.093	0.013	‡*	0.092	0.117	‡*	3
Courtney vs. Stavis	0.001*	0.100	0.001*	0.009*	‡*	‡*	0.103	0.001*	‡*	0.146	‡*	7
Courtney vs. Seabeck	‡*	0.006*	‡*	0.971	‡*	‡*	0.001*	‡*	‡*	0.384	‡*	8
Courtney vs. Big Beef	‡*	0.019	‡*	0.011	‡*	‡*	0.002	‡*	0.092	0.121	‡*	7
Courtney vs. Little Anderson	‡*	0.042	0.001*	0.011*	‡*	‡*	0.001*	0.010*	0.001*	0.253	‡*	8
Bear vs. Big	‡*	0.903	0.013	‡*	‡*	0.068	0.023	0.011	0.153	0.055	‡*	3

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Table 4.4 continued

Mission												
Bear vs. Stavis	0.007*	0.217	‡*	‡*	0.008*	‡*	0.132	0.001*	0.001*	0.225	‡*	7
Bear vs. Seabeck	‡*	0.162	0.001*	‡*	0.001*	‡*	0.001*	0.015*	0.002*	0.689	‡*	8
Bear vs. Big Beef	‡*	0.138	‡*	0.010*	0.002*	‡*	0.005*	0.033	0.077	0.115	‡*	6
Bear vs. Little Anderson	‡*	0.892	0.001*	0.001*	‡*	‡*	0.009*	0.004*	0.023	0.118	‡*	7
Big Mission vs. Stavis	‡*	0.124	0.003*	0.028	‡*	0.002*	0.008*	‡*	‡*	0.132	‡*	7
Big Mission vs. Seabeck	‡*	0.105	‡*	0.041	0.002*	‡*	0.006*	0.002*	‡*	0.004*	‡*	8
Big Mission vs. Big Beef	‡*	0.054	‡*	‡*	‡*	‡*	0.045	0.017	‡*	0.602	‡*	6
Big Mission vs. Little Anderson	‡*	1.000	‡*	0.008*	0.001*	‡*	‡*	0.001*	0.027	0.809	‡*	7
Stavis vs. Seabeck	‡*	0.029	‡*	0.002*	‡*	0.001*	‡*	‡*	0.745	0.172	‡*	7
Stavis vs. Big Beef	‡*	0.724	0.001*	0.267	0.001*	‡*	0.028	‡*	0.001*	0.349	‡*	6
Stavis vs. Little Anderson	‡*	0.048	‡*	0.682	0.001*	‡*	0.001*	‡*	0.136	0.153	‡*	6
Seabeck vs. Big Beef	0.001*	0.062	‡*	‡*	‡*	‡*	0.188	0.010*	0.003*	0.021	‡*	7
Seabeck vs. Little Anderson	0.001*	0.072	0.028	‡*	0.004*	‡*	‡*	‡*	0.138	0.014	‡*	6
Big Beef vs. Little Anderson	‡*	0.016	0.013	0.932	‡*	‡*	0.015	0.007*	‡*	0.730	‡*	5
*No. * (for 45 comparisons)*	42	14	40	24	40	41	27	41	20	10	45	-

‡ Indicates P < 0.0001.

* Indicates values judged significant with sequential Bonferroni adjustments for 10 simultaneous tests across loci (initial $\alpha = 0.05/10 = 0.005$).

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For the first distance matrix, tests for IBD indicated no correlation between geographic distance and θ_{ST}, CSE, or ρ_{ST} ($P = 0.065$, 0.244 and 0.402, respectively). However, using the second distance matrix, assuming Hood Canal was not a migration barrier, tests for IBD revealed highly significant correlations between geographic distance and both θ_{ST} and CSE, but not ρ_{ST} ($P = 0.015$, 0.001, and 0.50, respectively). These findings imply that Hood Canal does not represent a migration barrier to coastal cutthroat trout. Dendrogram relationships also support this view, with Thorndyke and Tarboo grouping more closely with the central Hood canal creeks than those from the southern cluster (see below; Figure 4.2). Using θ_{ST} and ρ_{ST} as measures of genetic distance, Wenburg et al. (1998, Chapter 3) did not find evidence for IBD among their 13 statewide populations. However, using the CSE matrix for genetic distance, there was a significant correlation with geographic distance among these populations ($P = 0.01$), indicating that IBD, while not as strongly supported as it is for populations within Hood Canal, appears to be at least loosely applicable to coastal cutthroat trout populations throughout Washington.

Overall for the nine anadromous populations (Fulton excluded) in Hood Canal, estimates of population subdivision as measured by θ_{ST} and ρ_{ST}, were 0.030 and 0.029, respectively (Table 4.5). ρ_{ST} values were significant for 31 of 36 pairwise comparisons ($P<0.05$) and in only one case did the 95% CI overlap zero. Although the significance of θ_{ST} values for this level of grouping was not tested directly, none of the 95% CIs for the 36 pairwise comparisons overlapped zero. When Fulton was included with the other nine

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Hood Canal populations, θ_{ST} and ρ_{ST} increased to 0.036 and 0.038, respectively, with all nine pairwise values being highly significant for both estimators (P<0.001) and lower limits of all 95% CIs being above zero. When combining the Yellowstone population with the nine anadromous Hood Canal populations (Fulton excluded), θ_{ST} and ρ_{ST} increased to 0.073 and 0.184, respectively. Again, all nine pairwise values for both of these estimators were highly significant (P<0.0001) and lower limits of the 95% CIs were well above zero.

Table 4.5. Summary of statistics for pairwise comparisons and various levels of grouping between coastal cutthroat trout populations using data from 10 microsatellite loci (8 loci for Yellowstone vs. Hood Canal grouping; Ots100, Ots103 excluded). *Nm* estimates were calculated for pairwise comparisons and the Hood Canal grouping only using the private alleles method (PR), and from θ_{ST} and ρ_{ST}, as described in the text. For each of the three groupings, 95% confidence intervals for ρ_{ST} and θ_{ST} and the mean of combined pairwise comparisons are given. Distance measure values are given for "CSE", the Cavalli-Sforza and Edwards' (1967) chord distance; "D", Nei's (1972) standard distance; and "$(\delta\mu)^2$", from Goldstein et al. (1995) for all pairwise comparisons, along with their mean values for the three groupings. Estimated time in years from the most recent common ancestor is given for the pairwise comparisons between populations within each grouping, along with the number of comparisons "*N*".

Comparison		*Nm* Estimates			Fixation Indices		Distance Measures		
		PR	θ_{ST}	ρ_{ST}	θ_{ST}	ρ_{ST}	CSE	D	$(\delta\mu)^2$
Thorndyke	Tarboo	2.8	4.6	3.2	0.052*	0.072*	0.04	0.15	3.56
Thorndyke	Fulton	0.8	1.9	1.5	0.115*	0.139*	0.10	0.39	9.12
Thorndyke	Courtney	1.6	3.6	7.9	0.065*	0.031*	0.05	0.19	1.14
Thorndyke	Bear	2.5	4.3	4.6	0.055*	0.051*	0.04	0.16	1.49
Thorndyke	Big Mission	2.7	5.0	4.7	0.047*	0.050*	0.04	0.14	2.50
Thorndyke	Stavis	2.7	7.1	3.2	0.034*	0.073*	0.03	0.11	4.33
Thorndyke	Seabeck	2.7	5.6	11.8	0.043*	0.021*	0.04	0.13	1.96
Thorndyke	Big Beef	2.9	10.2	5.1	0.024*	0.047*	0.03	0.09	2.06
Thorndyke	Little Anderson	2.0	5.1	6.3	0.046*	0.038*	0.03	0.13	1.19
Tarboo	Fulton	0.9	2.2	1.5	0.102*	0.147*	0.10	0.35	13.10

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Table 4.5 continued

Tarboo	Courtney	1.9	6.8	3.9	0.036*	0.061*	0.04	0.12	6.00
Tarboo	Bear	3.9	18.9	27.8	0.013*	0.009	0.03	0.07	1.05
Tarboo	Big Mission	2.8	7.2	3.9	0.034*	0.061*	0.04	0.11	3.34
Tarboo	Stavis	4.0	7.3	5.5	0.033*	0.043*	0.03	0.11	3.95
Tarboo	Seabeck	3.6	7.4	6.0	0.033*	0.040*	0.03	0.11	2.14
Tarboo	Big Beef	4.7	13.6	31.2	0.018*	0.008	0.03	0.08	1.07
Tarboo	Little Anderson	3.8	11.7	8.3	0.021*	0.029*	0.03	0.07	1.94
Fulton	Courtney	1.1	2.5	2.9	0.092*	0.079*	0.10	0.30	5.66
Fulton	Bear	1.1	2.2	1.7	0.103*	0.125*	0.10	0.35	8.91
Fulton	Big Mission	0.7	2.4	1.4	0.095*	0.150*	0.11	0.33	9.62
Fulton	Stavis	0.8	2.7	2.0	0.086*	0.112*	0.10	0.32	6.90
Fulton	Seabeck	0.8	2.4	1.8	0.095*	0.121*	0.10	0.33	8.44
Fulton	Big Beef	0.8	2.3	1.6	0.097*	0.136*	0.10	0.37	9.75
Fulton	Little Anderson	0.6	2.1	1.4	0.107*	0.152*	0.11	0.34	11.01
Courtney	Bear	3.6	11.6	8.6	0.021*	0.028*	0.03	0.09	2.50
Courtney	Big Mission	2.5	8.5	6.3	0.029*	0.038*	0.04	0.10	4.62
Courtney	Stavis	1.2	9.2	6.3	0.026*	0.038*	0.04	0.10	5.12
Courtney	Seabeck	1.7	5.5	7.9	0.044*	0.031*	0.04	0.14	3.83
Courtney	Big Beef	2.5	6.0	7.3	0.040*	0.033*	0.04	0.14	3.81
Courtney	Little Anderson	2.1	8.2	9.7	0.030*	0.025*	0.04	0.10	3.17
Bear	Big Mission	2.4	7.5	3.5	0.032*	0.067*	0.04	0.11	2.82
Bear	Stavis	2.5	8.2	5.6	0.029*	0.043*	0.03	0.11	2.93
Bear	Seabeck	3.2	6.2	8.7	0.039*	0.028*	0.03	0.13	1.57
Bear	Big Beef	2.9	9.7	20.8	0.025*	0.012	0.04	0.09	0.65
Bear	Little Anderson	2.5	9.9	8.0	0.025*	0.030*	0.04	0.08	1.26
Big Mission	Stavis	2.0	11.1	12.9	0.022*	0.019*	0.04	0.09	1.33
Big Mission	Seabeck	2.1	8.6	12.5	0.028*	0.020*	0.04	0.10	0.29
Big Mission	Big Beef	2.6	10.4	10.6	0.024*	0.023*	0.04	0.10	1.62
Big Mission	Little Anderson	2.5	11.4	40.9	0.021*	0.006	0.04	0.08	1.47
Stavis	Seabeck	2.0	7.4	15.3	0.033*	0.016*	0.03	0.11	0.97
Stavis	Big Beef	4.8	12.4	22.1	0.020*	0.011*	0.02	0.09	1.50
Stavis	Little Anderson	2.7	12.9	8.8	0.019*	0.028*	0.03	0.07	2.99
Seabeck	Big Beef	4.4	10.4	14.0	0.024*	0.018*	0.03	0.10	0.78
Seabeck	Little Anderson	5.7	8.9	14.7	0.027*	0.017*	0.02	0.09	0.94
Big Beef	Little Anderson	3.8	13.5	20.7	0.018*	0.012	0.03	0.07	1.19
Hood Canal (Fulton excluded)		6.0	8.4	8.3	0.030*	0.029*	-	-	-
N=9 0-10,000 years									
95% CI	Lower	-	10.2	7.7	0.024	0.031	-	-	-
	Upper	-	7.1	4.4	0.034	0.053	-	-	-
Mean of 36 pairwise comparisons:		-	-	-	0.031	0.033	0.025	0.166	1.95
Fulton vs. Hood Canal		-	-	-	0.036*	0.038*	-	-	-
N=10 10,000-15,000 years									
95% CI	Lower	-	-	-	0.031	0.038	-	-	-
	Upper	-	-	-	0.041	0.065	-	-	-
Mean of 9 pairwise comparions:		-	-	-	0.099	0.129	0.068	0.387	10.89

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Table 4.5 continued

Yellowstone vs. Hood Canal (Fulton excluded)	-	-	-	0.073*	0.184*	-	-	-
N=10 ca. 1,000,000 years								
95% CI Lower	-	-	-	0.054	0.181	-	-	-
Upper	-	-	-	0.100	0.211	-	-	-
Mean of 9 pairwise comparisons:	-	-	-	0.341	0.710	0.134	2.305	61.03

* Indicates significant θ_{ST} and ρ_{ST} values. For θ_{ST} pairwise comparisons, values were judged significant if 95% confidence intervals did not overlap zero, and for grouping comparisons, significance was determined directly permutation procedures (α=0.05/10=0.005 with sequential Bonferroni adjustments for 10 simultaneous tests across loci). Significance for all ρ_{ST} values were determined directly through permutation procedures using the same α level and adjustments.

The estimators, θ_{ST} and ρ_{ST}, were compared to each other by calculating the means, SDs and full range of 95% CIs for these values from the pairwise comparisons within each of three groupings. Within the Hood Canal grouping (Fulton excluded) the 95% CI ranges overlapped significantly (0.002-0.096 vs. -0.001-0.128, respectively) and their mean values were almost identical (0.031 (SD 0.012) vs. 0.033 (SD 0.018), respectively). For the Fulton vs. Hood Canal grouping, the 95% CI ranges continued to overlap (0.048-0.156 vs. 0.049-0.250, respectively) but their means were more disparate (0.099 (SD 0.009) vs. 0.129 (0.023), respectively). In the Yellowstone vs. Hood Canal grouping, the 95% CI ranges no longer overlapped one another (0.183-0.551 vs. 0.661-0.776, respectively) and the means were extremely disparate (0.341 (SD 0.018) vs. 0.710 (SD 0.021), respectively; Table 4.5; Figure 4.2).

GENE FLOW AND MIGRATION

Estimated numbers of effective migrants per generation, *Nm*, are given in Table 4.5 for the three indirect methods overall and for each pairwise comparison among the nine anadromous Hood Canal populations, along with their 95% CIs (for θ_{ST} and ρ_{ST}

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only). *Nm* values overall for Hood Canal derived from θ_{ST} and ρ_{ST} were very similar (8.4 and 8.3, respectively) and their 95% CIs overlapped (7.1-10.2 and 4.4-7.7, respectively), and they were only slightly higher than the value calculated with the private alleles method (6.0). Examining the various estimates from the pairwise comparisons shows more disparity between the methods, with no clear pattern emerging. Between the central Hood Canal cluster only, *Nm* estimates were 7.4, 10.6 and 15.6 for private alleles, θ_{ST} and ρ_{ST} methods, respectively (Table 4.6).

Table 4.6. *Nm* estimates for the four creeks in the central Hood Canal cluster. Values from 95-96 "Direct Count" given in parenthesis are counts including the two ambiguous migrants with dorsal fin CWTs (details in text). Ranges for 95-96 corrected *Nm* values were derived from calculations with and without these two ambiguous migrants.

Comparison	Indirect Methods			Direct Count		Corrected *Nm* from Direct Count	
	PR	θ_{ST}	ρ_{ST}	94-95	95-96	94-95	95-96
Big Beef vs. Stavis	4.8	12.4	22.1	1	5 (7)	4.7	23.0-32.1
Big Beef vs. Little Anderson	3.8	13.5	20.7	0	0 (2)	0	0-6.8
Big Beef vs. Seabeck	4.4	10.4	14.0	0	0 (2)	0	0-8.2
4 Creeks Combined	7.4	10.6	15.6	-	-	-	-

Table 4.7 gives the overall counts of smolt captured outmigrating from each of the four central Hood Canal creeks in 1994 and 1995 along with numbers marked with each type of tag, and weir down time expressed as a percentage of overall time of operation. Sixty-seven recaptures of fish originally tagged with CWTs (snout) and VI tags were made at Big Beef in 1995. Of these, 41 retained both tags, 21 retained the CWT only, and 5 retained the VI tag only. Probabilities of retention were therefore 0.61, 0.93 and 0.69 % for both tags, CWTs and VI tags, respectively, and the probability of losing both

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was 0.02%. The average number of days between tagging and recapture was 224 (N=43, range=1-381, SD=120.4).

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Table 4.7. Outmigration tagging summary by creek and year. "#", number of smolt counted; "#VI", number VI tagged, with percent of total in parenthesis; "#CWT", number CWTed, with percent of total in parenthesis; "#VI only", number VI tagged only; "#CWT only", number CWTed only; "% tagged", percent tagged with at least one tag; "% time weir down", and percent of sampling period weir was inoperable are given for each creek in 1994 and 1995.

	Big Beef		Stavis		Seabeck		Little Anderson	
	1994	1995	1994	1995	1994	1995	1994	1995
#	473	469	792	1052	148	229	50	340
# VI	437(0.92)	376(0.80)	672(0.85)	277(0.26)	129(0.87)	68(0.30)	36(0.72)	56(0.16)
# CWT	-	383(0.82)	-	750(0.71)	-	181(0.79)	-	328(0.96)
# VI only	446	14	683	3	129	0	36	2
# CWT only	0	22	0	479	0	113	0	274
% tagged	0.92	0.85	0.85	0.71	0.87	0.79	0.72	0.97
% time no weir	0.09	0.01	0.02	0.00	0.01	0.00	0.00	0.00

There were no confirmations of fish that outmigrated as smolt from Little Anderson or Seabeck that were later captured migrating into Big Beef as adults during the course of this study. However, there were two fish captured entering Big Beef with dorsal fin CWTs only, which therefore must have originated from one of these creeks or Stavis (the majority of smolt outmigrating from these creeks in 1995 had dorsal fin CWTs only and all Big Beef fish had snout CWTs). In 1995 there was one adult that migrated into Big Beef, which had outmigrated as a smolt from Stavis in 1994, and from 1995 to 1996 there were five. Only one of these strays was recaptured moving downstream in Big Beef. This migrant was in an obvious spawned out condition, and presumably strayed into and spawned in Big Beef. However, the other five strays were not recaptured on their way downstream and therefore it was not possible to assess whether or not they actually spawned. These fish either passed downstream during weir down time, remained in fresh water until at least mid-June or else did not survive to return to the ocean due to spawning mortality, predation etc.

To account for the two ambiguous strays with dorsal fin CWTs only, a range of *Nm* estimates were calculated from the direct observations between Stavis and Big Beef for 1995-1996. Values of five and seven were substituted for M in Equation 1. This yielded direct *Nm* estimates of 23.0-32.1 in 1995-1996 (Table 4.6). Further, assuming that the two migrants with dorsal fin CWTs were alternatively from Little Anderson and Seabeck, similar ranges of estimates for *Nm* were calculated between these creeks and Big Beef from 1995 to 1996 from 0-8.2 (Table 4.6).

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DISCUSSION

POPULATION STRUCTURE AND GENE FLOW IN HOOD CANAL

These results suggest that all populations analyzed are genetically distinct; that is, independent creeks in Hood Canal form distinct breeding units for coastal cutthroat trout. Tests of genotypic differentiation revealed significant heterogeneity among all populations. While the level of differentiation, measured by number of differentiated loci, increased with the geographic distance between populations, even adjacent creeks separated by as little as 2 km exhibited significant differences in genotype frequencies at five of ten loci (Big Beef and Little Anderson). Moreover, values for ρ_{ST} were significant in 31 of 36 pairwise tests between populations and in only one case did the 95% CIs for ρ_{ST} or θ_{ST} overlap zero. Similarly, Wenburg et al. (1998, Chapter 3) found significant allele frequency heterogeneity among all 13 coastal cutthroat trout populations they analyzed and Campton and Utter (1987) determined that stream effects explained the greatest degree of allelic frequency variation found between populations in their study.

Courtney and Bear creeks were the least diverged populations in this study, differing significantly in genotype frequencies at only one locus. However, the combined test over all loci rejected the null hypothesis of genotypic homogeneity and pairwise values for θ_{ST} and ρ_{ST} were significant, with 95% CIs that did not overlap zero. These creeks are separated by only 4 km and were the only ones sampled in this study that are tributaries to a larger river. As such, they may be expected *a priori* to be the most similar. However, as was found by Campton and Utter (1987), it appears that even

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on this level distinct breeding units may exist in this subspecies, and at the level of independent creeks flowing directly into salt water the evidence for genetic heterogeneity is unequivocal.

In light of the relatively small overall geographic area covered in this study, the degree of population substructuring found here notable among anadromous salmonids (see Chapter 3, Table 3.8 for comparisons between several studies). These values are below those obtained for some non-anadromous populations such as brook char (*Salvelinus fontinalis*) (Angers et al. 1995) and brown trout (*Salmo trutta*) (Ryman 1983), but are comparable to those reported from microsatellite and allozyme studies among other anadromous salmonids including coastal cutthroat trout (Campton and Utter 1987; Wenburg et al. 1998, Chapter 3), Atlantic salmon (Sanchez et al. 1996; McConnell et al. 1997), sockeye salmon (*O. nerka*) (Ryman 1983) and steelhead (Ryman 1983). Moreover, the values obtained here for θ_{ST} and ρ_{ST} (0.030 and 0.029, respectively) among nine coastal cutthroat trout populations in Hood Canal, all within a 100 km area, were higher than those typically reported for pink salmon across large geographic ranges. For example, Olsen et al. (1998) reported lower values for θ_{ST} and ρ_{ST} from 12 odd-year pink salmon populations spanning from Alaska to Washington (0.022 and 0.026, respectively). This example also exemplifies a circumstance where similar F_{ST} values, and therefore *Nm* estimates derived from them, can be obtained from presumably very different effective population sizes and migration rates (see Allendorf and Phelps 1981).

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Direct estimates for *Nm* between Stavis and Big Beef varied substantially across the two years of this study. Further, under the assumption that the two strays with dorsal fin CWTs in 1996 were from Stavis, there were no recorded strays into Big Beef from Little Anderson or Seabeck. However, based on indirect estimates, the number of strays between Big Beef and each of the three adjacent creeks were roughly equivalent. Taken together these results suggest that straying in coastal cutthroat trout may be highly stochastic in nature. Studies attempting to directly observe migrational events on relatively short time scales, such as the present one, may therefore be inadequate to characterize the true degree of straying among coastal cutthroat trout populations. In addition, the potential logistical difficulties encountered (e.g., flood events in 1994-1995) and the relatively low numbers of migrational events can make accurate quantification from direct observation problematic.

Indirect estimates of *Nm* from F_{ST} analogous involve several simplifying assumptions which are likely not met in natural populations. Two of the most important assumptions being the infinite island model of population structure and equilibrium between migration and genetic drift. It has been shown that coastal cutthroat trout populations tend to be structured in an IBD fashion where immigrants are more likely to come from nearby populations, invalidating the infinite island model assumption. However, Slatkin (1985) and Slatkin and Barton (1989) showed that both the infinite allele and F_{ST} methods for estimating *Nm* values are robust enough to provide reasonable estimates over a wide range of conditions, even when the island model assumptions are

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violated. The infinite stepping-stone model provides the greatest possible isolation by distance, and as such may be more accurate in describing coastal cutthroat trout population structure. This model effectively lies on the opposite end of a theoretical continuum of possible population structure models from the island model. Comparing results of simulation studies using assumptions from these alternate models which represent diametrically opposed extremes, Slatkin and Barton (1989) showed that F_{ST} and infinite alleles estimations of *Nm* provided comparable and realistic results. Further, especially for small populations, equilibrium is quickly reached between genetic drift and migration, estimated to be on the order of $1/m$ years (Slatkin 1985). Nonetheless, *Nm* estimates are just that, estimates, and as Slatkin (1985) asserts, realistically in surveys of natural populations, values are probably "accurate only to within a factor of two".

The range of *Nm* between Stavis and Big Beef from direct estimates from 1995-1996 is slightly above that for the indirect estimates, but for all other years and creeks the direct estimates are close to or slightly lower than the indirect estimates (Table 4.6). In a similar type study of chum salmon (*O. keta*), Tallman and Healey (1994) found indirect gene flow estimates from allozyme data to be well below those indicated by mark-recapture methods. Similarly, Quinn et al. (1987) found the level of gene flow inferred from allozyme data to be much lower than levels estimated from direct assessment using naturally occurring parasites as "tags" in sockeye salmon (*O. nerka*). Both studies concluded that the discrepancy was best explained through differential reproductive success of straying and homing fish, and Tallman and Healey (1994) suggest that this

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"selection" may be a powerful force in maintaining stock discreteness. While such heavy selection against strays undoubtedly occurs under certain circumstances, the general homogeneity among and close proximity of the adjacent creeks in the current study renders it likely that there would be a relatively small differential of reproductive success between straying and homing trout on this spatial scale, which may at least partially explain the lack of disparity between the direct and indirect estimates.

It should also be noted that Tallman and Healey (1994) used samples from returning adults for their genetic analysis to determine *Nm* estimates between streams. These samples were collected in a different year from the tagging experiment and may have contained strays which could not be identified as such, confounding the interpretation of *Nm* estimates. In the present study, this potential bias was avoided through sampling only outmigrating smolt. This further avoided a the potential bias from sampling anadromous species such as the coastal cutthroat trout which, as shown here in Chapter 5 and by Michael (1989), may migrate in and out of one or more creeks before entering their spawning creek. They have also been shown to overwinter in non-natal creeks (Michael 1989), similar to the anadromous Dolly Varden (*Salvelinus malma*) (Bernard et al. 1995). Obviously, sampling of adults in such species may lead to an unwanted admixture of populations and should be interpreted with caution. Although there may also be some bias introduced when sampling juveniles (see Allendorf and Phelps 1981), for anadromous populations in species with complex migration patterns such as these it may be preferable to sampling of adults, especially if sampling can be

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designed to capture juvenile or smolt over protracted spatial or temporal scales to avoid the inclusion of related individuals. This type of sampling scheme was employed in the present study, and was at least partially validated by the relative absence of gametic and genotypic disequilibrium in these populations.

While both direct and indirect methods of gene flow estimation involve numerous simplifying assumptions, what is striking from the present study is that both methods provided independent estimates that were on average quite similar. The empirical data indicate that both estimates appear to yield biologically realistic estimates of gene flow between coastal cutthroat trout populations. So, while the underlying assumptions may not always be met, it appears that indirect models are nonetheless robust enough to provide meaningful approximations of gene flow, at least when applied to realistic situations where gene flow is not precluded due to the known isolation of populations. Moreover, they may be more easily obtained due to the logistical difficulties for studies employing direct methods over sufficient time scales in wild salmonids.

As indicated by estimates of gene flow between populations and the support found for IBD, persistent gene flow is likely occurring among coastal cutthroat trout populations. The higher significance for IBD within Hood Canal versus that found in the statewide populations combined with the limited saltwater migrations of coastal cutthroat trout (see Wenburg et al. 1998, Chapter 3) indicate that gene flow is strongest within regions, but is nonetheless occurring between regions. Indeed, straying at some level must occur between regions throughout the range to account for the original colonization

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following glacial retreat, and is a "strategy" for which the evolutionary payoff has been substantial. However, the existence of persistent straying between populations must not be taken as an indicator of panmixia. Migration between populations does not preclude genetic differentiation. Although the theory has been inappropriately applied (see Mills and Allendorf 1996), the occurrence of one migrant per generation (OMPG) is not sufficient to preclude differentiation between populations, but is merely sufficient to ensure the same alleles will be shared over long periods of evolutionary time (Allendorf and Phelps 1981). Indeed, significant allelic divergence will often occur when there is substantial exchange among populations (Allendorf and Phelps 1981), which is exactly what was found in the present study: highly differentiated populations with straying rates well in excess of OMPG. In fact, taken together, the methods employed here yielded estimates on the order of 10 migrants between populations per generation. Interestingly this is the theoretical ideal level of gene flow, proposed by Mills and Allendorf (1996), between populations for artificial implementation between fragmented populations in a conservation context.

POPULATION STRUCTURE AND GENE FLOW THROUGHOUT WASHINGTON

Coastal cutthroat trout populations throughout Washington show strong subdivision with nearly all creeks and streams studied to date comprising distinct breeding units (Campton and Utter 1987; Wenburg et al. 1998, Chapter 3; present study). Straying between populations within regions maintains some genetic similarity between them that decreases with increasing geographic distance but does not preclude the

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formation and maintenance of genetically isolated populations. Similarly, some straying between regions occurs, likely being strongest between ecologically similar regions. And as suggested by Wenburg et al. (1998, Chapter 3) large-scale relationships between various regions may reflect ecological heterogeneity between them and possibly the residual effects of historical dispersal from multiple refugium.

Although bootstrap values were low, as expected with the relatively low numbers of microsatellite loci used here (Takezaki and Nei 1996), with a few anomalous exceptions, the overall relationships between the 23 combined populations (Figure 4.2) follow *a priori* expectations based on their geographic proximity and the limited distance and duration of saltwater migrations for coastal cutthroat trout (see Wenburg et al. 1998, Chapter 3). The tight clustering of all Hood Canal populations and relatively short branch lengths between them indicate that relationships are strong within ecological homogenous regions. However, it appears that the strongest contributor to population structure in coastal cutthroat trout is differentiation at the level of individual creeks and streams, overriding the formation of disjunct regional groupings. Similar results were found by Fontaine et al. (1997) for Atlantic salmon (*Salmo salar*) populations in Quebec, Canada, where stream effects were found to be most significant level of structuring, overriding the effect of regional groupings.

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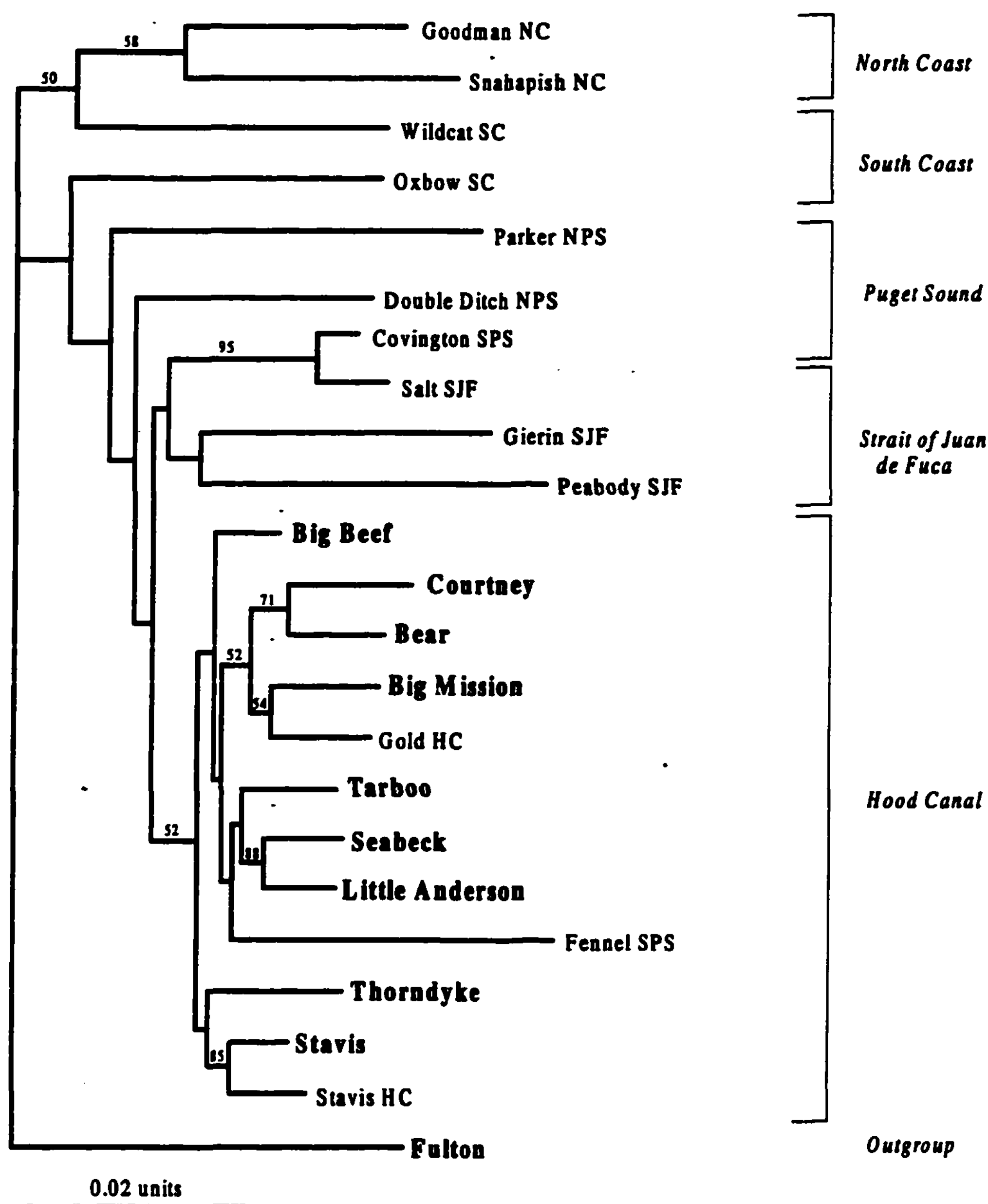

Figure 4.2 Neighbor-joining dendrogram for pairwise CSE distances among 23 coastal cutthroat trout populations constructed with data from six microsatellite loci. Data for populations in bold are from this chapter; data for all others are from Wenburg et al. (1998, Chapter 3).

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MICROSATELLITE PROPERTIES

While microsatellites are widely considered one of the most powerful genetic markers in use today, several aspects of their mutational properties remain unresolved, which call into question their applicability for various investigations and the appropriateness of the statistical methods used in their analysis. The major concerns in this arena are the appropriateness of the mutational models employed, range constraints on allelic sizes, homoplasy between populations and the apparent lack of linearity with time for distance measures (see Takezaki and Nei 1996 and Goldstein and Pollock 1997 for review and detailed discussions of each). It has been shown both through simulation (Takezaki and Nei 1996) and with empirical evidence (Paetkau et al. 1997) that for the restricted time scales of intraspecific population studies, where genetic drift is considered the primary force driving genetic changes, the degree of accuracy in the mutational models employed (e.g., IAM vs. SMM) and the degree of linearity with time for genetic distance measures is of less importance than the sample variance of those measures. The remaining issues, however, remain of concern for studies on the intraspecific level. While this study was not designed to specifically address these issues, samples from populations with known evolutionary relationships allowed a re-examination of the data with these issues in mind in order to evaluate the performance of various genetic statistics.

According to Behnke (1992) divergence between ancestors of the Yellowstone cutthroat and the coastal cutthroat trout occurred approximately 1 million years ago. The

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non-anadromous population, Fulton, was collected from above a barrier to migration formed approximately 10,000-15,000 thousand years ago as the glaciers receded from the Hood Canal region. At about this same time, predecessors to the present day anadromous Hood Canal populations were allowed to disperse from their glacial refugia, likely in a Puget Sound lake (Thorson 1980), and spread outward along the coast. Therefore, by testing various groupings of these populations their divergence on three time scales can be explored: 1) between the anadromous Hood Canal populations (36 pairwise comparisons; ca. 0-15,000 years; 2) between the anadromous Hood Canal populations and the non-anadromous Fulton population (nine pairwise comparisons; ca. 10,000-15,000 years); and 3) between the nine anadromous Hood Canal populations and the Yellowstone cutthroat population (nine pairwise comparisons; ca. 1,000,000 years). Although sample sizes for the Fulton and Yellowstone populations were relatively small, comparing the properties of various distance measures and F_{ST} analogues at these three levels may shed some light on the forces shaping the spatial distribution of microsatellite allele frequencies and the subsequent applicability of various statistics.

The overall similarities of allele sizes and ranges between all the populations analyzed here were striking, especially when considering the divergence times between them. For the Hood Canal and statewide populations combined, Fulton contained a total of only four private alleles at two loci (three at Oneμ2, one at Ots100) which fell just outside the size range for alleles in the anadromous populations (Appendix E). Similarly, but even more striking, the Yellowstone population, after 1,000,000 years of divergence,

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exhibited only six private alleles at two loci (one at Omy77, five at Oneμ2), and at only one locus (Oneμ2) did these alleles fall outside the size range of those present in the Hood Canal populations. Although the possibility of one-way gene flow from Fulton into the anadromous Hood Canal populations could not be ruled out, this level of overlap strongly suggests the existence of range constraints and/or drastically varying mutation rates between loci in cutthroat trout. The high numbers of alleles per locus and high mutation rates assumed to be associated with them make it unlikely that low and varying mutation rates alone could account for this lack of divergence, but instead indicate strong range constraints on allelic size ranges at microsatellite loci. Subsequently, assuming the existence of range constraints, high mutation rates, and the passing of hundreds to thousands of generations, homoplasy (co-occurrence of alleles that are identical-in-state though not identical-by-descent) becomes an almost certainty. Angers and Bernatchez (1997) found extensive homoplasy between and even within salmonid species resulting from various mutational events, and Estoup et al. (1995) found homoplasy to be frequent at the subspecies level in bees. The data reported here lend further circumstantial support for the existence of range constraints and homoplasy at microsatellite loci by at least the subspecies level. This may strongly affect the degree of linearity with time for all genetic distance measures, reinforcing the need for a new class of statistics for microsatellites that account for these properties (Goldstein and Pollock 1997).

If the three distance measures used here (CSE, D, and $(\delta\mu)^2$) increased linearly with time and proceeded roughly under a SMM, one would conservatively expect that the

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mean distance between the Yellowstone population and the anadromous Hood Canal populations to be approximately 66-100 times that between Fulton and the Hood Canal populations. Previous study and simulation (Takezaki and Nei 1996; Paetkau et al. 1997) has shown that among these distance measures, CSE is the first to lose linearity with time, followed by D and finally $(\delta\mu)^2$. Paetkau et al. (1997) estimated that even $(\delta\mu)^2$ begins to lose linearity at the interspecific level and possibly at the intraspecific level, specifically at less than 20,000 years of separation. These inferences were drawn from microsatellite analysis in bears (*Ursus spp.*) using an estimated generation time of 10-15 years, or about 1,300-2,000 generations. Therefore, among cutthroat, using an average generation time of 3 years, the time of separation at which even the most robust measures begin to plateau could be as little as 4,000-6,000 years, assuming that mutation rates and size constraints are similar between taxa. The mean of the distances among Fulton and the Hood Canal populations was higher than that among the Hood Canal populations alone, for CSE, D and $(\delta\mu)^2$, the ratios being 2.7, 2.33 and 5.6, respectively (Table 4.5). The ratios between the Yellowstone vs. Hood Canal grouping and the Fulton vs. Hood Canal grouping were only 2.0, 6.0 and 5.6, respectively, well below the expected ratio of 66-100 assuming linearity with time. All ratios were at least an order of magnitude below the expected values providing further evidence that the linearity of these measures with time plateaus somewhere below the subspecies level, likely due to allelic range constraints.

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Direct comparison of θ_{ST} and ρ_{ST} values may yield insight into the forces shaping population subdivision. The mutational processes of microsatellites do not erase all information about the ancestral allelic state, as mutations tend to at least roughly proceed following a SMM (but see also Di Rienzo et al. 1994 and Angers and Bernatchez 1997 and references therein), which is taken into account for ρ_{ST} calculations. However, θ_{ST} values do not take this added information into account, but instead focus on the degree of overlap between allele frequency distributions, which obviously cannot decrease below zero. θ_{ST}, therefore, tends to overestimate genetic similarity and underestimate coalescence times, the degree to which this occurs being positively correlated with divergence between populations and the allelic variability of the loci involved. As coalescence times decrease, the performance of θ_{ST} improves because genetic drift becomes the more dominant process creating local differentiation, and mutational events are of less importance. Comparison of θ_{ST} and ρ_{ST} values from empirical data may highlight the point at which the importance of mutation exceeds that of genetic drift in the formation of allele frequency distribution between populations. Using the population groupings above, the means of the pairwise θ_{ST} and ρ_{ST} values among the Hood Canal populations were very similar with extensively overlapping 95% CI ranges. This suggests that these populations are of relatively recent evolutionary origin, and that drift and migration have predominated over mutation in shaping the pattern of genetic differentiation. At the intermediate level, among Fulton and Hood Canal populations, the mean of the pairwise values for ρ_{ST} became larger than θ_{ST}, but their 95% CI ranges

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continued to overlap. However, at the Yellowstone vs. Hood Canal level, the mean value for ρ_{ST} was significantly higher with no overlap in the 95% CI ranges (Table 4.5; Figure 4.3). It therefore appears that for cutthroat trout, as one approaches the subspecies level, the historical information from distances between alleles increases in importance such that, if it is ignored, there will be drastic underestimation as to the degree of population structure. The differences between the mutational models upon which these estimators are based begin to become important at the intraspecific level (ca. 3,000-5,000 generations) and provide drastically different results by the subspecies level (up to 300,000 generations).

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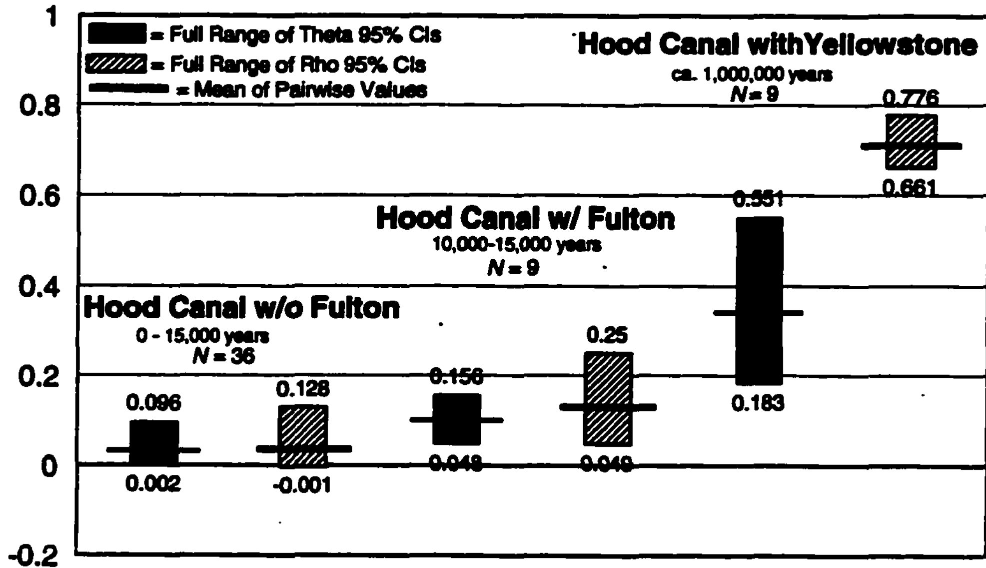

Figure 4.3 Comparison of means for pairwise values of θ_{ST} (Theta) and ρ_{ST} (Rho) for population groupings at three time scales. Highest and lowest values for 95% CIs are given for all pairwise tests at each level of grouping.

In general, the results from this study were consistent with those of other researchers in regard to varying mutation rates, constraints on allele size and the tendency for the linearity with time of distance measures to plateau off below the species level of divergence. While many of the parameters estimated through the available statistics are robust enough to provide meaningful results for most population level studies, the unique properties of microsatellites and the apparent variability between loci seem to lend themselves to categorization by class based on allelic size range, variability and mutation

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rate, as suggested by Goldstein and Pollock (1997). Further, if future research shows that allelic variance is an adequate predictor of these variables, then selection of loci from various classes may be dictated *a priori* by the question being asked or the hypothesis being tested (Goldstein and Pollock 1997).

CONCLUSIONS

Synthesizing the genetic and migration data from this study and Wenburg et al. (1998, Chapter 3), it appears that the basic genetic unit in the population structure of sea-run cutthroat trout is the individual creek. This conclusion is supported by the results of the extensive genetic analysis reported in these studies, most notably those from statistical tests for genetic heterogeneity and subpopulation structure among populations from creeks throughout Hood Canal and the entire state of Washington. This study also provided strong evidence for the occurrence of reproductive straying between adjacent creeks in Hood Canal, which, as has also been shown here, does not preclude genetic differentiation between them. In general, the comparison of gene flow estimates from direct and indirect methods provided similar results, validating the applicability of both methodologies. Finally, these data provided further circumstantial support for the occurrence of size range constraints and homoplasy at microsatellite loci, at or below the subspecies level, as well as rough estimates for the time scales at which these properties become important for various statistical parameters.

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CHAPTER 5: LIFE HISTORY TRAITS AND ECOLOGY OF THE COASTAL CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI CLARKI*) IN HOOD CANAL, WASHINGTON

INTRODUCTION

Within the cutthroat trout complex, the coastal cutthroat (*O. clarki clarki*) is the most abundant and widely distributed of the 14 currently recognized subspecies, exhibiting a wide array of complex life history strategies (Behnke 1992). The range of the sea-run cutthroat trout extends from the Eel River in California, USA, to Gore Point on the Kenai Peninsula in Alaska, USA, with inland penetration generally limited to less than 150 km (Behnke 1992), which conforms closely to the coastal temperate rain-forest belt as defined by Waring and Franklin (1979). Populations of coastal cutthroat trout exhibit four basic life history forms: resident, fluvial, adfluvial, and anadromous. Unless otherwise noted, this chapter will address the anadromous form only, hereafter referred to as the sea-run cutthroat trout.

Sea-run cutthroat trout have undergone a major range-wide decline over the past two decades (Nehlsen et al. 1991; Trotter et al. 1993), although data are limited and locally some populations remain healthy. The American Fisheries Society Endangered Species Committee concluded that all native naturally spawning populations in the states of California, Oregon and Washington are at some level of risk, being either of special concern or on the threshold of becoming threatened or endangered (Nehlsen et al. 1991). The California Department of Fish and Game has classified the subspecies as a "Species

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of Special Concern" (Gerstung 1997), in Oregon they are currently listed on the "Sensitive Species List" (Hooton 1997), and in Washington, although there appears to be a mix of healthy and depressed populations, many populations are in a state of steady decline (Leider 1997). In addition, the United States National Marine Fisheries Service (NMFS) recently listed all forms of the coastal cutthroat trout of the North Umpqua River in Oregon as endangered pursuant to the Endangered Species Act of 1973 (ESA) (NMFS 1996).

The decline of sea-run cutthroat trout populations throughout their native range has led to concern over the limited ecological information available for this subspecies. Even details on much of the basic ecology of sea-run cutthroat trout are severely limited (Trotter et al. 1993) as relatively little research has been performed on this subspecies. Much of the information available was gathered as incidental information from activities targeting other more economically important *Oncorhynchus* species (Leider 1997). In an effort to rectify this paucity of information and in anticipation of future petitioning to list more coastal cutthroat trout populations as threatened or endangered, NMFS is currently conducting a coastwide review for coastal cutthroat trout status and ecological requirements in an effort to designate ESUs throughout their range (NOAA 1994a). Similarly, the Washington Department of Fish and Wildlife (WDFW) recently initiated a stock inventory and status assessment process for all coastal cutthroat trout (Leider 1997). Genetic analyses have been initiated as part of these assessments and were addressed and previously reported here in Chapters 3 and 4. However, details on

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migration patterns, habitat usage and much of the general ecology of sea-run cutthroat trout remain uncertain, providing incomplete information on which to base conservation and management decisions (Trotter et al. 1993).

Sea-run cutthroat trout tend to spawn in small low-gradient watersheds or in tributaries in the lower reaches of larger systems. Juveniles rear in fresh water for one to six years, with most fish smolting at age two, three or four, depending on locality. It has been suggested that physical and biological characteristics of the marine environment into which the smolt enter may exert selective pressures on smolt age and size (Johnston 1982; see also Chapter 3, Discussion). Seaward movements begin in March and continue through May. In the relatively protected marine environment of Puget Sound, smolt are predominantly age two (Leider 1997), with those emigrating to the more exposed surf zones of coastal areas being predominantly age four (Fuss 1982).

Sea-run cutthroat trout migrations are relatively limited, although very little is known about the actual movement details. In general, populations are believed to remain near river mouths and estuarine areas and not to migrate to the open ocean or cross large bodies of open water (Johnston 1982; Jones 1976) as is common with anadromous populations of other *Oncorhynchus* species (Groot and Margolis 1991). Overall, sea-run cutthroat trout migrations are relatively short, both temporally and spatially, being generally limited to less than six months and less than 100 km (Giger 1972; Johnston 1982; Jones 1976) whereas migrations of hundreds to thousands of kilometers over periods up to six years or more are common among other *Oncorhynchus* species (Groot

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and Margolis 1991). Some sea-run cutthroat trout populations may even spend their entire saltwater residence period within the confines of a single river estuary (Tomasson 1978). It is assumed that sea-run cutthroat trout seldom if ever overwinter completely at sea (Trotter 1989), however some limited evidence suggests that they may (Johnston 1982).

The return migration timing of sea-run cutthroat trout to fresh water varies by locality. Populations are generally classified as early or late-entry, as defined by Johnston and Mercer (1976). The former enter fresh water as early as July and continue through November, and are usually associated with major river systems. The latter are generally associated with small independent drainages and enter fresh water from December through March. Individual and sex-specific patterns of migration have not been previously investigated.

The cutthroat trout complex is one of three in the genus *Oncorhynchus* within which species are iteroparous. Details of spawning activity for sea-run cutthroat trout are similar to other salmonids, often taking place in tailouts under 15 to 45 cm of water over a period of 2-3 days (Trotter 1997). Not all sea-run cutthroat trout will spawn during their first return migration into fresh water. Those that do not are said to be on "feeding runs", the proportion of which reportedly varies by population, and is highest among first-time migrants and females. This behavior also tends to vary with geographic area with reports ranging from 5% (Sumner 1952) to nearly 50% (Tipping 1981) of upstream migrants being feeding run fish. The term feeding run, however, may be somewhat of a

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misnomer as some populations may feed very little if at all upon their return to fresh water (Giger 1972).). Homing to natal creeks for spawning is believed to be very precise in sea-run cutthroat trout (Campton and Utter 1987). However, complex migration patterns have been observed, including at least physical straying of sea-run cutthroat trout into non-natal creeks (see also Chapter 4). For clarity, a distinction is made here between physical straying, that is, merely migration into a non-natal creek, and reproductive straying, whereby a physically straying fish also reproduces in a non-natal creek.

Few details of the overwintering period of sea-run cutthroat trout have been previously documented (Trotter et al. 1993; Trotter 1997). Weight loss of up to 38% of body weight may occur (Sumner 1953) and survival estimates ranging from approximately 10-50% have been reported (details and references given below). The return of spawned-out adults and feeding run fish to salt water tends to peak in March and April (Trotter 1997). These migrations tend to precede the seaward migration of sea-run cutthroat trout smolt and other juvenile salmonids and may as such be favored by selection, as it places the adults in a position to intercept this potential food source in estuaries (Johnston 1982).

This study was designed in an effort to augment currently available information; data is presented and analyzed here from a three year tag-recapture study to address several aspects of sea-run cutthroat trout ecology. The movement and condition of sea-run cutthroat trout was monitored past a series of weirs on four adjacent creeks in Hood Canal, Washington over the course of three seasons. Individual tracking and scale

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analysis of a large subsample of migrants allowed the precise determination migration timing for individuals into and out of fresh water over the course of one to three seasons. In addition, the hypotheses that differential migration patterns, habitat usage and reproductive strategies exist between the sexes were tested, and various survival and age structure parameters were determined for these populations. The sea-run cutthroat trout populations in the creeks studied here are relatively pristine, they are wild and native and there are currently no releases of hatchery fish in this area. Further, the populations studied here are currently protected from sport-fish harvest through Wild Cutthroat Release regulations (Leider 1997).

METHODS

Using the permanent weir facility located at the mouth of Big Beef Creek in Hood Canal, WA, and temporary weirs on three adjacent creeks, migrant sea-run cutthroat trout were sampled, tagged and monitored between 1993 and 1996. Specific details on the dates and times of weir operation for all creeks in each year are given in Chapter 4. Briefly, the weir at Big Beef Creek was maintained to capture migrants in both directions almost continually from August 5, 1993 – June 12, 1996, except for infrequent flood events and periodical maintenance and during the summer months of 1994. It should, however, be noted that there were several major flood events during the 1994-1995 season. The first of these caused major damage to the weir that forced it to be opened continuously for 17 days. This occurred during a time when significant upstream migrations were expected. Indeed, the upstream passage of large numbers of undetected

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migrants was confirmed during that spring when two more adults were captured moving downstream than had been captured moving upstream during that season. This is especially significant when one considers the typical mortality rate during the freshwater migration period, ca. 50% (see below), which indicated that around half of the upstream migration was missed during this season. As such, many of the results from this year are suspect and are so noted in presentation of results and discussion. Both smolt and adults were captured, sampled and tagged throughout this period as detailed in Chapter 4. Temporary weir traps were constructed during the spring outmigration periods only (March or April through June) at Stavis, Seabeck and Little Anderson creeks in order to sample and tag emigrants from these creeks.

Scale samples were collected from a subsample of smolt and adults. These samples were pressed and read following standard procedures by John G. Sneva of the Washington Department of Fish and Wildlife (WDFW). Sea-run cutthroat trout scales are notoriously difficult to age (Howard Fuss and John G. Sneva, WDFW, personal communications). In this study several scale samples were collected across years for tagged individuals with known migration and spawning histories, providing validation of scale feature interpretation, most notably the occurrence of spawning checks, thus improving the accuracy of analysis. Notation followed that currently in use at WDFW: smolt ages were the number of freshwater annuli; adult ages were designated by the smolt age prior to the decimal, following the decimal a "+" indicating partial years and separated post-smolt annuli, an "F" indicating a non-spawning annulus (either a feeding

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run or a winter spent at sea), and an "S" indicating a spawning annulus; "R" indicating a regenerated scale for which age could not be estimated, and an "R." indicating a partially regenerated scale which could not be assigned a smolt age, but could be assigned a post-smolt age.

Standard statistical procedures were used for the various parameters analyzed in this study; specific tests and relevant values are reported along with results. Where appropriate, *F*-Tests were used to test for equal variances between samples (results not shown) before subsequent *t*-tests. Two-tailed tests and an α level of 0.05 were used throughout to judge significance of results.

RESULTS AND DISCUSSION

There were a total of 598 capture events for adults at Big Beef Creek over the course of this study with 373 of these involving fish which were captured more than once (included in these numbers are 41 smolt outmigration tagging events, including 5 from Stavis, for fish later recaptured as adults at Big Beef). One-hundred and seven fish were captured twice, and 19, 11, 1 and 1 were captured three, four, five and six times, respectively. Numbers of smolt outmigrating from each of the three creeks and the numbers tagged during this study are given in Chapter 4 (Table 4.7). These data do not include migrant parr which passed downstream in varying numbers throughout the study and were not tagged. These fish were generally much smaller (<100 mm) than smolt and did not exhibit the silver coloration and body shape typical of smolting fish. Migrating

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parr have been reported by others in similar studies (Sumner 1962; Giger 1972). The significance of these migrants and their effect on their respective populations is unknown.

The mean sizes of adult migrants returning to fresh water increased significantly for each successive migration, as determined through scale analysis (ANOVA: $P<0.001$; Table 5.1). Mean fork lengths for 1st and 2nd return fish in this population were higher than those reported by Michael (1989) for Snow and Salmon creeks in Washington (1st = 303 mm, range 203-438, N=43; 2nd = 402, range 263-485, N=17), by Johnston (1979, cited in Michael 1989) for the Stillaguamish River, also in WA (1st = 276 mm, range 155-356, N=88; 2nd = 361, range 305-420, N=12), and by Sumner (1962) for Sand Creek in OR (1st = 317.5 mm, N=376; 2nd = 370, N=122).

The populations in this study, and those from Snow, Salmon and Sand creeks are considered late-entry, with the Stillaguamish population being an early-entry stock. Given the longer saltwater residence of late-entry stocks, it is not surprising that they tended to be larger than early-entry stocks for a given return migration. However, as can be seen from the comparison of Big Beef length-at-return information with that from Snow and Salmon creeks, there is significant variation even within stock types. This variation exemplifies the need to obtain population, or at the very least, region specific data for length-at-return estimates. Management plans often rely on the accuracy of such data. For instance, current WA fishing regulations are designed to ensure that juveniles and pre-smolt are protected and that most adults are able to spawn at least once prior to harvest (Leider 1997). Depending on the degree of protection desired, harvest

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restrictions on sea-run cutthroat trout from Hood Canal populations would have to be much more conservative in terms of minimum sizes in order to account for the relatively large size of these fish at first return to fresh water.

Table 5.1 Fork length (mm) at various life history stages for sea-run cutthroat trout in Big Beef Creek. Return number was determined through scale analysis. Results are given for 1st return migrants overall and for the subsamples of these fish determined to have smolted at ages 2 and age 3, and overall only for 2nd, 3rd, and 4th time return migrants.

Stat.	1st Return FL			2nd Return	3rd Return	4th Return
	Overall	Age 2 smolt	Age 3 Smolt	FL	FL	FL
Mean	342	342	350	406	429	452
SE	3.2	4.2	10.2	6.2	19.7	-
Range	169-421	205-400	244-420	312-473	378-485	-
N	171	69	18	40	5	1

Upstream migration into Big Beef Creek began as early as October 15 in 1993 and as late as October 27 in 1994 (Figure 5.1, Table 5.2). The latest individual upstream migration occurred on April 25 in 1994. The month with the heaviest upstream migration varied by year and sex, ranging from November to March. For example, during the 1993-1994 season the months of heaviest migration were December and March for males and females, respectively, and during the 1994-1995 season the heaviest migration occurred in February for both sexes. On average, males arrived in fresh water sooner

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than females (Figure 5.1, Table 5.2). The 50% point of the upstream migration was reached sooner by males in all three years of the study. In addition, *t*-tests (sample sizes given in Table 5.5) confirmed that the mean of the upstream migration dates (standardized to day of the year) varied significantly between males and females during the 1993-1994 and 1995-1995 seasons (*P*=0.002 and 0.005, respectively). There was not a significant difference detected in migration timing between the sexes for the 1994-1995 season (*P*=0.97), however this was likely due to the extended period of weir down time during this season detailed above. Most importantly here, the weir was inoperable from December 19, 1994 through January 4, 1995. For the other two years of the study, 11% and 16% of the total upstream migration of males occurred during this same time period as compared to 6% and 10% for females. It therefore appears likely that a larger proportion of the undetected migrants during the early part of this season were males. This, along with the highly significant results obtained for upstream migration date differences between the sexes in the other two years of the study, indicates that the upstream migration analysis was likely significantly biased for the 1994-1995 season.

The timing of the downstream migration out of Big Beef Creek was much less variable and occurred over a much shorter period of time than the upstream migration (Figure 5.1, Table 5.2). The earliest downstream migration was January 23 in 1994, and the latest was May 25 in 1995. In all years for both sexes, the majority of downstream migration occurred in April, the 50% mark for the downstream migration was reached between April 3-8, and the last migration occurred in May. Downstream migration dates

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(standardized to day of the year) did not vary between the sexes during the 1993-1994 or 1995-1996 seasons (*t*-tests, P=0.10 and 0.93, respectively), but did vary significantly during the 1994-1995 season (P=0.03).

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Sea-run Cutthroat Trout Migration

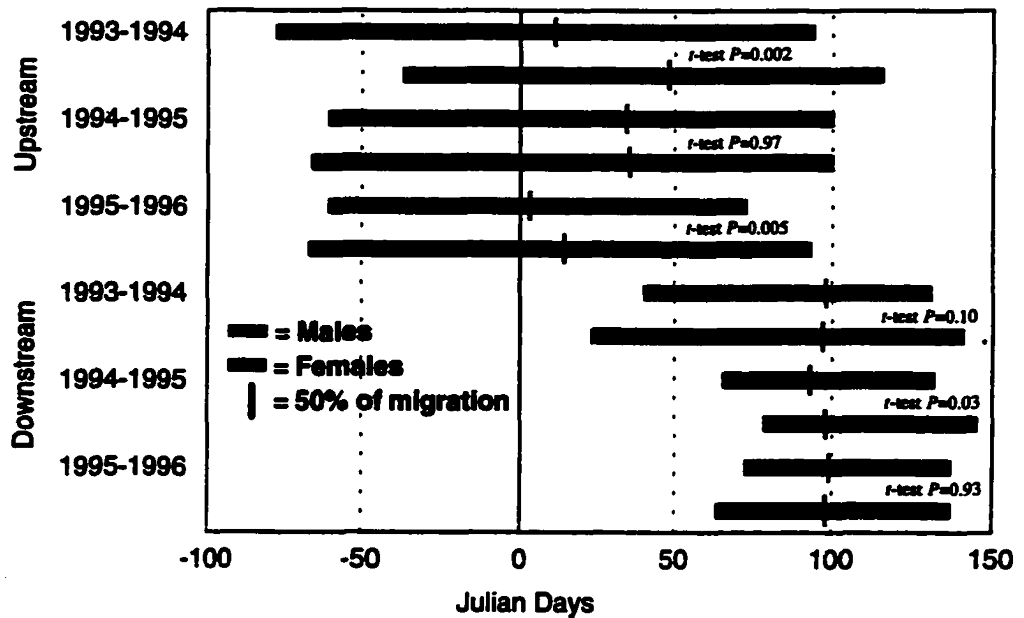

Figure 5.1 Sea-run cutthroat trout migration by day of the year where January 1st =1. Duration of both upstream and downstream migrations by sex are given for each of three years, along with date at which 50% of each migration was reached and results of *t*-tests between the sexes for each year and direction. Data from Table 5.2.

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Table 5.2 Migration timing for adults at Big Beef Creek. Dates are given by sex for date the first migrant was captured "1st", date on which 50% of the run had passed the weir "50%", date the last migrant was captured "Last", and the month of heaviest migration "Month" and the percent of each run that passed the weir during that month "%" in both directions for each of three years. See also Figure 5.1.

Year	Sex	Upstream				Downstream			
		1st	50%	Last	Month (%)	1st	50%	Last	Month(%)
1993 -1994	*Male*	Oct.15	Jan.11	April 4	Dec.(32)	Feb.9	April 8	May 11	April(70)
	Female	Nov.24	Feb.17	April 25	March(32)	Jan.23	April 7	May 21	April(55)
1994 -1995	*Male*	Nov.1	Feb.3	April 10	Feb.(48)	March 7	April 3	May 12	April(57)
	Female	Oct.27	Feb.4	April 10	Feb.(46)	March 20	April 8	May 25	April(75)
1995 -1996	*Male*	Nov.1	Jan.3	March 13	Nov./Jan.(37)	March 13	April 8	May 16	April(74)
	Female	Oct.26	Jan.14	April 2	Jan.(29)	March 4	April 7	May 16	April(66)

As detailed in Chapter 4, for the three creeks adjacent to Big Beef Creek (Stavis, Seabeck and Little Anderson), trapping of downstream migrants typically spanned from March or April into June, as the main purpose was the capture of outmigrating smolt. As such, only partial counts for outmigrating adults were obtained for these three creeks. However, if it is assumed that similar proportions of adults from the four creeks returned to salt water at the same time in each year, extrapolation from the more complete Big Beef Creek data can be used to estimate the total run sizes for the other three creeks. For each creek, in each year, the percent of the Big Beef run which had outmigrated by the time their respective weir was in place was calculated, then corrected the number

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captured after that point, assuming the two were identical in proportion, to estimate the total number of downstream adult migrants. An average survival of 48% (1993-1994 in Table 5.5, see below) was then assumed, to estimate the total run size for each creek (Table 5.3).

Table 5.3 Adult downstream migrants from Stavis, Seabeck, and Little Anderson creeks. Given are the direct counts, extrapolated number of migrants assuming equivalent and proportional downstream migration timing with Big Beef Creek, and estimated total run sizes assuming 48% survival from 1993-1994 Big Beef Creek survival data.

Creek	Downstream Adult Counts		Extrapolated # Downstream Adults		Estimated Total Run Size	
	1994	1995	1994	1995	1994	1995
Stavis	39	34	51	57	106	119
Seabeck	8	13	11	19	23	40
Little Anderson	6	8	8	50	17	104

SCALE ANALYSIS

The aging for many of the sea-run cutthroat trout samples collected was complicated by relatively high levels of regenerated scales. Approximately 25% of the scales samples from Big Beef adults were not readable, and 50% of those for which post-

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smolt ages could be determined were partially regenerated precluding assignment of pre-smolt age. Similarly, approximately 25% of the 311 smolt samples from Big Beef (*N*=260) and Stavis (*N*=51) were not reliably readable.

However, reliable ages were determined for 200 smolt outmigrating from Big Beef Creek (Appendix F). These samples consisted of individuals ages 1-3, with each age class differing significantly in mean size (ANOVA, $P<0.001$) but with overlapping size ranges. The mean size for age one smolt was 126.8 mm (SE 15.5), ranging from 102-186 (*N*=5), age two smolt had a mean size of 170.0 mm (SE 2.9), ranging from 110-277 (*N*=162), and age three smolt had a mean size of 190.7 mm (SE 4.8), ranging from 143-266 (*N*=33). Thirty-one Stavis smolt samples were determined to be age two, with a mean size of only 133.8 mm (SE 2.2), which differed significantly from the Big Beef age two smolt (*t-test*: $P<0.001$). This difference was likely to be at least partially a result of the presumably increased productivity and warmer water temperatures in Big Beef Creek associated with William Symington Lake, a 198-ha artificial impoundment constructed 10 km upstream from the weir in 1965.

A total of 218 scale samples from Big Beef adult migrants were read reliably for post-smolt ages, with 108 of these also being assigned pre-smolt ages (Table 5.4). Seventy-eight percent (*N*=171) of these adults were first time migrants, with the remaining 22% being on their second (*N*=41), third (*N*=5) or fourth (*N*=1) freshwater migration. Of the 47 repeat migrants, 40 (85%) were female, which is significantly more

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than expected, even considering that overall 64% of the aged adults were female (χ^2 test: $P<0.005$). All adults determined to have pre-smolt ages of one or four were males ($N=6$).

Table 5.4 Ages from scale analysis for adult sea-run cutthroat trout from Big Beef Creek. Pre- and post-smolt ages are given for each year and combined for all three years, with numbers of females given in parenthesis.

Big Beef Creek	1993-1994	1994-1995	1995-1996	Total
N	76 (51)	65 (44)	77 (45)	218 (140)
Pre Smolt:				
Age 1	-	1 (-)	1 (-)	2 (-)
Age 2	23 (14)	22 (17)	41 (26)	86 (57)
Age 3	7 (5)	6 (4)	6 (1)	19 (10)
Age 4	-	1 (-)	-	1 (-)
Post Smolt:*				
R.	46 (32)	35 (23)	29 (18)	110 (73)
.+	70 (45)	43 (25)	58 (30)	171 (100)
.+S+	5 (5)	10 (9)	10 (7)	25 (21)
.+F+	1 (1)	11 (10)	4 (4)	16 (15)
.+S+S+	-	1 (-)	2 (2)	3 (2)
.+F+S+	-	-	2 (1)	2 (1)
.+F+S+S+	-	-	1 (1)	1 (1)

*Post-smolt codes are as follows: samples for which post-smolt ages only were determined "R."; first time migrants ".+"; second time migrants that spawned the previous season ".+S+"; second time migrants that were on a feeding run, or overwintered at sea,

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Table 5.4 continued

the previous season but did not spawn ".+F+"; third time migrants that spawned in the previous two seasons ".+S+S+"; third time migrants that were on a feeding runs, or overwintering at sea, and spawning runs for their first and second migrations, respectively ".+F+S+"; and fourth time migrants that were on a feeding run, or overwintering at sea, for their first migration and spawning runs for their second and third ".+F+S+S+".

OVERWINTERING IN FRESH WATER

Overall survival estimates for adults during the upstream freshwater migration period in Big Beef Creek for the three years of this study are given in Table 5.5. These values are calculated from overall numbers of migrants and not from individual tag data, with the following exception. As the majority of migrants were tagged and thus individually identifiable, several fish were noted migrating in either or both directions multiple times within a season. To avoid artificially inflating numbers of migrants, these fish were counted only once in each direction. During the 1993-1994 season, the weir was considered "fish tight" except for two overnight flood events where water passed over the top of the weir for approximately 1 hour (< 0.001%). For the 1994-1995 season, there were several flood events which likely allowed for significant numbers of undetected migrants. As highlighted above, the dates of these flood events, especially the first two, coincided with times of expected upstream migration (Dec. 19, 1994-Jan. 4, 1995, Feb. 16, 1995-Feb. 20, 1995, Feb. 26, 1995-March 3, 1995, and March 9, 1995-March 15, 1995; total = 744 hours ≈ 10%). It is likely that the upstream counts were therefore most effected in this year, causing the overall numbers of migrants to be

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underestimated, and conversely causing survival estimates to be artificially inflated. Indeed, this was reflected in the survival estimates for the 1994-1995 season which exceeded 100% (Table 5.5). Although to a much lesser degree, the reverse is true for data from the 1995-1996 season, when weir down times predominantly coincided with expected downstream migration periods (10 hours total from Jan. 21, 1996-Feb. 7, 1996, 1 hour on March 11, 1996, 8 hours each for March 19, 1996-March 23, 1996, and April 23, 1996-April 25, 1996; total = 110 hours ≈ 2%) likely causing the number of downstream migrants, and therefore survival estimates, to be slightly underestimated. While they are likely to vary with environmental conditions, the survival estimate for the 1993-1994 season of 48% is likely the most accurate. These values are similar to those previously reported for sea-run cutthroat trout overwintering survival (22.2 – 76.9%, Michael 1989; 23-79% Washington State Department of Fisheries, unpublished report; 32% Sumner 1952; 11-46% Sumner 1962). In this study, survival rates for females were slightly higher than for males, but the differences were not significant over the three years of this study (*t-test*: P=0.93).

Table 5.5 Counts, sex ratios, and overwinter survival estimates for adult migrants overwintering in Big Beef Creek.

Year	Males		Females		Upstream	Combined		% over winter survival		
	Up	Down	Up	Down	Ratio M:F	Up	Down	Males	Females	Combined
1993-1994	73	33	116	58	1:1.6	189	91	0.45	0.50	0.48
1994-1995	31	30	52	55	1:1.7	83* (177)	85	0.97	1.06	1.02
1995-1996	49	19	96	32	1:2.0	145	51	0.39	0.33	0.35

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Table 5.5 continued

Total	153	82	264	145	1:1.7	417*(511)	227	0.54	0.55	0.54

*Major flood events caused extensive weir down time mainly during the upstream migration period for this season (details in text). The numbers in parenthesis are estimates based on a 48% survival rate and the count of 85 surviving downstream migrants.

Individual identification and recapture of migrants allowed me to analyze the movements and condition of 104 adults (67 females and 37 males) overwintering in Big Beef Creek over the course of this study. Each of these fish was captured migrating upstream into, and downstream out of Big Beef Creek, during the same season. On average, males spent significantly more time in fresh water than did females (71 and 53 days, respectively; *t*-test: P=0.01), and tended to arrive earlier (detailed above). Condition factor [K=100(weight in g)/(fork length in cm)^3] was noted for 98 of these migrants both upon entering and exiting Big Beef Creek. The average decrease in K per day was significantly higher for females (N=57) than for males (N=29) (*t-test*: $P<0.01$), but the overall decrease in K did not differ significantly between the sexes (0.20 and 0.25, respectively; *t-test*: P=0.14). Similarly, for 86 of these migrants, the average loss in grams per day was significantly higher in females (mean=4.1, N=57) than in males (mean=1.9, N= 29; *t*-test: P=0.002), but the overall weight loss was not significantly different between the sexes (100 and 136, respectively; *t-test*: P=0.12). The average weight loss in terms of % of body weight was 21.7% (SE 0.018) for males and 23.1% (SE 0.013) for females and were not significantly different (*t*-test: P=0.53). Only two fish gained weight while in fresh water (8 g in 54 days; 34 g in 61 days), suggesting that

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lengthy freshwater residence is energetically costly for adult sea-run cutthroat trout, presumably even for years in which they do not spawn, and that feeding is limited during freshwater residence. Ignoring these two fish, the range of % body weight lost was 5.9-42.2% in males and 2.0-44.4% in females. It is possible that it is the winter season which is costly to sea-run cutthroat trout and not freshwater residence itself as there is no information available on the change in condition for fish overwintering in salt water. Nonetheless, as very few if any sea-run cutthroat trout remain at sea during the entire winter (but see below), it may be hypothesized that the fitness costs of freshwater overwintering may be lower than those associated with of remaining in salt water, beyond the obvious cost to reproductive fitness of missed spawning opportunities for mature fish remaining in salt water. Little is known about the ecology of sea-run cutthroat trout in salt water, but possibilities for increased costs include decreased winter food supply, predation (Giger 1972), and varying degrees of saltwater tolerance (Johnston 1982).

While the overwintering mortality did not differ significantly between the sexes, it appears they employed alternate migration strategies and experienced differential energetic costs associated with them, which nonetheless led to similar outcomes. It has been shown in this study that, on average, males spent 34% more time in fresh water than females, and tended to arrive sooner. This is consistent with previous study of *Oncorhynchus* species showing the importance to males of prior residency and the establishment of spawning territories centered around females and/or high quality nest sites, both of which increase their access to females (Foote 1990). In addition, as with all

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Oncorhynchus species, males have the ability to spawn multiple times with one or more females. Therefore, by arriving sooner and remaining on the spawning grounds longer, males tend to increase their reproductive opportunities. In contrast, females, which only spawn once during a season, tended to arrive in fresh water later, emigrate after fewer days, and lose more weight during the spawning migration, presumably due to egg deposition. Spending less time in fresh water potentially provided them additional feeding opportunities in salt water (see Holtby and Healey 1990), presumably increasing their survival, and fecundity, by aiding them in the recovery from the rigors of spawning. While males expended less energy in the spawning act *per se*, and increased their reproductive fitness by remaining on the spawning grounds longer to potentially mate with more females, they presumably had increased energetic costs associated with longer freshwater residence and direct intrasexual spawning competition, which can be intense (Foote et al. 1990; Quinn et al. 1996). The outcome for each of these alternate strategies was similar, in that mortality rates were near 50% for both sexes (Table 5.5).

The sex ratio of upstream migrants was heavily in favor of females, with a male:female ratio of 1:1.7 (Table 5.5), similar to the 1:1.6 ratio reported sea-run cutthroat trout by Sumner (1952). The tendency for males to remain on the spawning grounds in fresh water longer would effectively bring the sex ratio closer to 1:1, as would the occurrence of resident precocious males. Precocious resident male cutthroat as small as 100 mm have been reported (Sumner 1952), and indeed were intermittently captured in this study (data not shown). However, the proportion of such spawners has not been

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systematically investigated in sea-run cutthroat trout, although they have been shown to be high in other *Oncorhynchus* species such as the Atlantic salmon (*Salmo salar*).

For individuals of both sexes, linear regression analysis revealed a strong negative correlation between the number of days spent in fresh water and the date on which they entered (males: $R^2 = 0.89$, ANOVA $P<0.0001$, Figure 5.2; females: $R^2 = 0.57$, ANOVA $P<0.0001$, Figure 5.3). That is, those adults which entered fresh water earlier tended to spend more days in fresh water. These results are also consistent with the findings above that males arrived in fresh water sooner and stayed longer, and that downstream migrations tended to occur at about the same time across years for both sexes. Further suggesting that there is an optimum time for migrants of both sexes to leave fresh water, which is independent of when they arrive.

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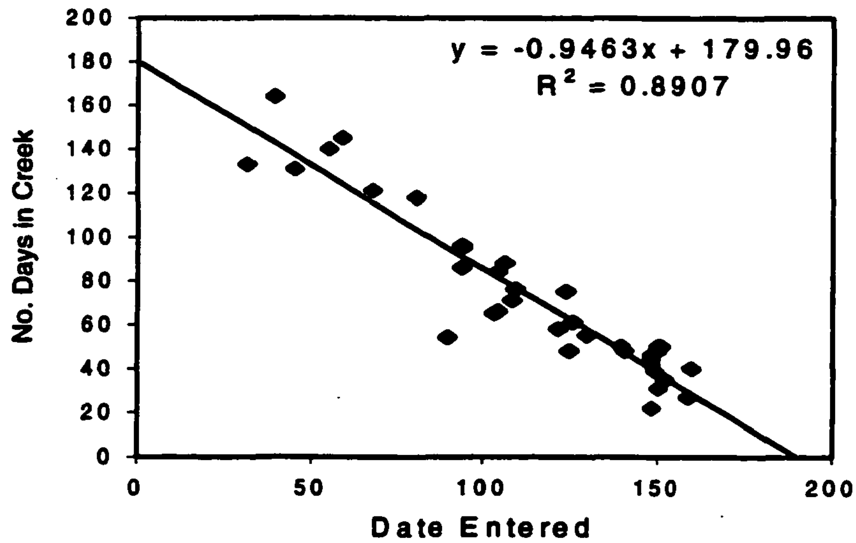

Figure 5.2 Regression of number of days spent in fresh water on date of entry into fresh water (October 1st = 0) for 37 male sea-run cutthroat trout in Big Beef Creek from 1993-1996.

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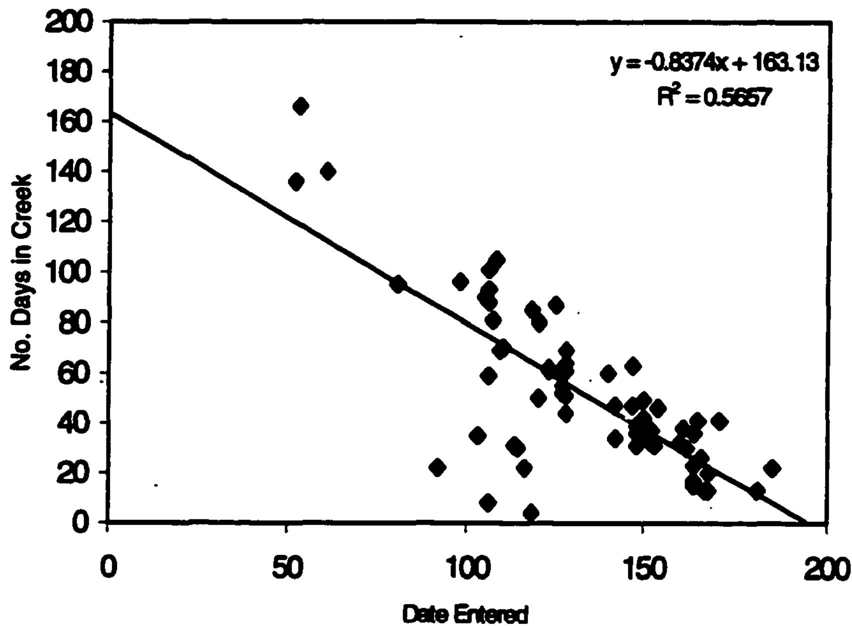

Figure 5.3 Regression of number of days spent in fresh water on date of entry into fresh water (October 1st = 0) for 67 female sea-run cutthroat trout in Big Beef Creek from 1993-1996.

MIGRATIONS INTO SALT WATER

Using percentages of first time migrants as determined through scale analysis, total numbers of upstream migrants, and subsequent numbers of outmigrants, survival estimates were calculated for smolt to 1st freshwater migration in all three years for Big Beef (Table 5.6). These calculations assumed: 1) the age structure of the subsample of aged individuals were representative of the population, 2) all migrants returned to fresh water each season, and 3) all migrants returned to their natal creek, or alternatively that for each fish straying from Big Beef, there was another which strayed into Big Beef of

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the same age class. The salt water survival estimates here (11.6-23.2%) were comparable to those found in previous studies. Michael (1989) estimated marine survival from smolt to first return ranged from 1.8 – 21.7% in two creeks less than 100 km from Hood Canal, Giger (1972) reported marine survivals of 20-40% for Oregon stocks, and Jones (1978) reported survival in an over-harvested Alaskan stock at 17%.

Using counts of adults surviving to migrate back downstream in 1993 and 1994, adjusted for the percent of those runs that determined through scale analysis to be first-time migrants, and adjusting the number of upstream migrants in the following years for the percentages determined through scale analysis to be second-time migrants, survival rates between first and second upstream migration in 1995 and 1996 were calculated (Table 5.6). These survival rates between first and second upstream migration (33.3-58.9%) were similar to the 39% previously reported for Sand Creek, an Oregon stock, also in the absence of a fishery (Sumner 1952).

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Table 5.6 Saltwater migration survival estimates for sea-run cutthroat trout from Big Beef Creek. Estimates are given from smolt to first return, for three years, and from outmigration after first return to second return, for two years.

Smolt		Adult Migrants					Adjusted Total 1st Return	Adjusted Total 2nd Return	Survival % Smolt to 1st Return	Survival % 1st to 2nd Return
Year	Count	Year	Count Up	Count Down	1st Return	2nd Return				
1993	727	1993-1994	189	91	0.92	-	174	-	0.239	-
1994	473	1994-1995	83	85	0.66	0.34	55	28	0.116*	0.333*
1995	469	1995-1996	145	-	-	0.23	109	33	0.232	0.589

*These values are likely underestimated due to the several flood events during expected upstream migration times during the winter of 1994-1995 (details in text).

The movements of 48 tagged adults (34 females and 14 males) were observed and analyzed during the saltwater portion of their life cycle. Each of these fish were captured in the spring migrating downstream from Big Beef Creek into salt water and re-captured the following fall/winter upon re-entering Big Beef. On average, males spent less time in salt water than did females, although the difference was not significant (255 (SE 11.3) vs. 269 (SE 11.5) days, respectively; *t*-test: P=0.44). Condition factor was noted for 38 of these migrants both upon exiting and re-entering Big Beef. The average increase in K was again higher for males (N=9) than for females (N=29) but was not significant, both overall (0.20 (SE 0.04) and 0.18 (SE 0.02), respectively; *t*-test: P=0.68) and by day (7.8×10^{-4} and 6.7×10^{-4}, respectively; *t*-test: P=0.28). Similarly, the average weight gain

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in grams per day was higher in males (1.6 (SE 0.14), N=9) than in females (1.3 (SE 0.22), N= 29), but again the differences were not significant (t-test: P=0.34). However, the average overall weight gain was slightly higher, but not significantly so, in females (413 (SE 15.4) g, N=27) than in males (409 (SE 35.2) g, N=9; t-test: P=0.85). Growth in length was higher for males than females, both overall (123 (SE 12.4) and 102 (9.2) mm, respectively) and by day (0.50 (SE 0.06) and 0.37 (0.03) mm, respectively), but again the differences were not significant (P=0.31 and 0.11, respectively). The differences between the sexes in average weight gain in salt water, calculated as % body weight, were not significant, being 154% (SE 0.19), ranging from 78-216%, for males, and 135% (SE 0.15), ranging from 59-297%, for females (P=0.48).

As noted above, it has been suggested that some sea-run cutthroat trout may overwinter at sea (Johnston 1982). Bernard et al. (1995) concluded that this was indeed the case for large numbers of anadromous Dolly Varden (*Salvelinus malma*) which show strong similarities to sea-run cutthroat trout in several aspects of their life histories and migration patterns. There were seven tagged cutthroat in this study which were seen in 1994 and 1996 but not in 1995, all of which were females. As there were times during the 1994-1995 season that the weir at Big Beef was inoperable, it is possible that these fish did return, but went undetected in 1995. Scale analysis was performed for one of these females which was captured leaving Big Beef on May 4, 1994 and not seen again until it passed back upstream on January 3, 1996. Scale analysis indicated that this fish had not spawned during the 1994-1995 season. There is no definitive way to tell from scale

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analysis if a fish made a feeding run into fresh water or spent that time in salt water (John G. Sneva, WDFW, personal communication). Therefore, this fish may have remained at sea for 609 days, or entered and exited Big Beef on a feeding run during 1995 undetected, or strayed to a different creek for a feeding run during the 1995 season. However, these data indicate that indeed some sea-run cutthroat trout overwintered at sea in this study.

STRAYING

It is common among *Oncorhynchus* species for some reproductive straying to occur between populations, the extent of which appears to vary by species, population and even environmental conditions between years (Ricker 1972). There have been no documented cases of reproductive straying among wild sea-run cutthroat trout. Giger (1972) reported significant physical straying among hatchery stocks of sea-run cutthroat trout, but such behavior among hatchery and transplanted stocks of salmonids does not provide a reliable estimate for wild stocks (Ricker 1972). Jones (1975, 1976) and Michael (1989) also reported physical straying in wild populations of sea-run cutthroat trout among predominantly immature feeding run fish, but reported no evidence for reproductive straying. Similarly, extensive physical straying has been observed in Dolly Varden along with little or no evidence of reproductive straying (Armstrong 1970, 1974, Bernard et al. 1995). Although it could not be determined definitively as actual spawning events were not witnessed, there is strong evidence to support the existence of both physical and reproductive straying among sea-run cutthroat trout populations. Ten fish were captured in more than one creek during the course of this study (details in Appendix

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G for the following VI tagged fish: T68, TK4, T08, Z38, AF9, E33, EH3, F04, and two others with dorsal fin coded wire tags only for which individual identification was not available). Seven outmigrating smolt were later recaptured at least once in a creek other than the one that they originally outmigrated from, and hence presumably their native creek (all creeks in question empty directly into salt water). Two of these were captured leaving their presumed non-natal creeks as adults in a spawned out condition, and the other five were clearly of reproductive size and appeared to be developmentally ready to spawn upon migrating upstream into non-natal creeks. Further, one of these was captured migrating upstream into the same non-natal creek in two consecutive years in a fully mature and ripe condition. These data indicate that some reproductive straying does occur among the creeks of Hood Canal, a conclusion that is further supported by the genetic analysis reported here in Chapter 4.

Feeding Run Estimates

Percentages of sea-run cutthroat trout migrants on feeding runs have been estimated from 5 to 50% for different populations (Jones 1972, 1973, 1974, 1975, 1976; Johnston 1982; Tipping 1981; Fuss 1982). The estimates in this study fell towards the low end of this range. As noted above, there is no definitive way to tell from scale analysis if a fish made a feeding run into fresh water or spent the winter in salt water. However, scale analysis indicated that a maximum of 38 out of 218 (17%) aged adults had engaged in feeding runs, that is if it is assumed none of these fish overwintered at sea. This is likely an overestimation, for as detailed above, it appears likely that at least

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some of these migrants spent the entire year at sea. In addition, although there is no objective criteria on which to base designations, individual weight loss information combined with visual assessment indicated that throughout this study, more than 90% of upstream migrants were spawners.

Although much of the variation in feeding run percentages may be explicable by inter-population differences, there may be another factor involved. In this study, some upstream migrants entered fresh water up to several months prior to spawning. Results from individual identification confirmed that from visual assessment alone as fish passed upstream, especially for the earlier migrants, it was not always possible to correctly determine if fish were going to spawn during that migration. As most studies must make such visual assessments of upstream migrants only, as fish are usually not individually identifiable, it appears likely that in such studies the proportional estimates of spawning fish may be underestimated as eventual spawners may appear "green" upon entering fresh water, and thus be misclassified as feeding run fish.

MIGRATION DATE FIDELITY

Timing for upstream migration (Gharrett and Smoker 1993), spawning time (Siitonen and Gall 1989), and age at maturity (Hankin et al. 1993) have been shown to be heritable and therefore under some degree of genetic control in other *Oncorhynchus* species; however, to my knowledge the question of repeat migration date fidelity in iteroparous individuals has not been investigated. There were 51 individuals that were captured migrating downstream (*N*=32) or upstream (*N*=19) in more than one year. This

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allowed me to investigate the question, Do individuals tend to migrate upstream or downstream on similar dates across years? The results indicated that there was no apparent correlation between the date of downstream migration (standardized for day of the year) across years for individuals ($R^2 = 0.05$, ANOVA P=0.22, N=32, data not shown). However, with the removal of a single extreme point, regression analysis confirmed that there was a significant correlation between the upstream migration date in one year for an individual and the upstream migration date in the next year for that same individual ($R^2 = 0.43$, ANOVA P=0.002, N=19, Figure 5.4).

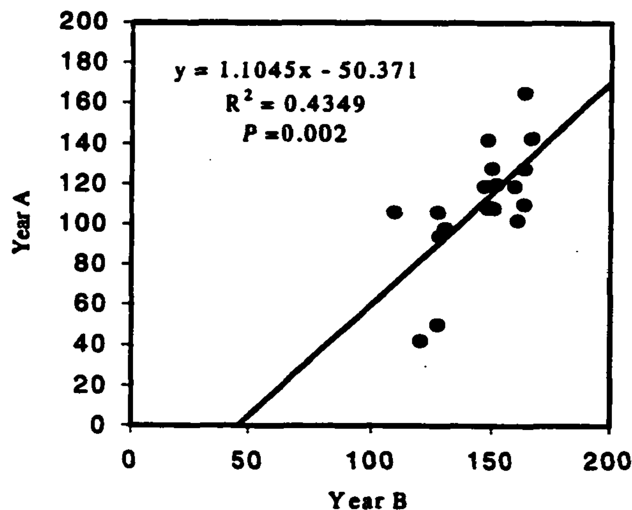

Figure 5.4 Regression for date of upstream migration (October 1st = 0) in one year on upstream migration in the next year for 19 sea-run cutthroat trout into Big Beef Creek. One data point (44,148), was removed as an outlier for regression analysis.

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Conclusions

This chapter reported on a three year ecological tag-recapture study of native sea-run cutthroat trout populations in Hood Canal, Washington. These results are likely among the most accurate to date, as these populations are protected from sport fish harvest and that the identification of individuals in this study allowed for more accurate estimates than those typically available from other studies. Survival rates for the main study population, Big Beef Creek, both for the freshwater and saltwater migration periods, and the proportion of repeat spawners were high relative to the few results previously reported. It was also demonstrated that males tended to migrate into fresh water sooner than females and stayed longer, and that there is some fidelity to the upstream migration date for individuals across years. And finally, the strongest behavioral evidence to date was reported, indicating that sea-run cutthroat trout engage in reproductive straying between creeks and that some migrants remain at sea during the entire overwintering period. It is my hope that these data, along with the genetic analyses of the previous chapters, will be of use in the conservation efforts currently underway, and those likely to follow, in the attempt to preserve and protect the coastal cutthroat trout.

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APPENDIX A: REPRINT OF COMPANION PAPER TO CHAPTER 2.

Molecular Marine Biology and Biotechnology (1996) 5(4), 259–272

Semiautomated multilocus genotyping of Pacific salmon (*Oncorhnychus* spp.) using microsatellites

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Abstract

We report the development of a semiautomated multilocus genotyping system for Pacific salmon using four-color fluorescent detection of microsatellites. An initial screening of microsatellites was conducted on five species of Pacific salmon (*Oncorhynchus* spp.) and Atlantic salmon (*Salmo salar*) using 35 primer pairs developed from six species of salmonid. The number of loci that amplified varied by species from 11 (chum salmon) to 22 (chinook salmon). We then tested co-amplification of microsatellites in chinook, coho, and sockeye salmon and developed six-locus multiplex systems. The species-specific multiplex systems were applied to two populations using a sequencer/gene scanner (Perkin-Elmer Applied Biosystems, Inc. [ABI] 373A). The genetic variability at each locus was calculated to evaluate the utility of this system for genetic studies. Significant differences in allele frequencies were observed between populations in 14 of 18 pairwise comparisons. Average heterozygosity ranged from 0.47 in Togiak River coho salmon to 0.75 in Dungeness River chinook salmon. Observed heterozygosities ranged from 0 at Oneμ1 in Togiak River coho to 0.96 at Ssa85 in Dungeness River chinook. The probability of match (P_M) for each six-locus multiplex system was 4.0×10^{-10}, 7.2×10^{-6}, and 3.2×10^{-7} for chinook, coho, and sockeye, respectively. The average polymorphic information content (PIC) was 0.77, 0.56, and 0.60 for chinook, coho, and sockeye, respectively. The microsatellite loci used here show promise for high-resolution genetic studies of Pacific salmon such as fine-scale population analysis kinship, and parentage studies.

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Introduction

Genetic markers have been used extensively for management and conservation of Pacific salmon (*Oncorhynchus* spp.) (Allendorf et al., 1987; Carvalho and Hauser, 1994; Utter, 1994). Protein electrophoresis is the primary method used to detect genetic population structure among discrete spawning groups and estimate stock composition in mixed stocked fisheries (Utter et al., 1987). However, three factors have contributed to an interest in applying new DNA-based genetic markers. First, in some cases protein-coding loci lack sufficient polymorphism to reveal fine-scale genetic population structure (Gyllensten and Wilson, 1987; Currens et al., 1994; Forbes et al., 1994; Tessier et al., 1995). Second, protein electrophoresis requires lethal sampling and immediate cold storage (−40° to −80°C) of tissue samples, which limits its feasibility in threatened or endangered species and imposes logistic difficulties in sampling collection and storage, respectively. Finally, the combination of nonlethal sampling and extensive polymorphism detectable with DNA markers facilitates novel applications such as kinship analysis, the use of DNA profiles as genetic "tags," and marker-assisted selective breeding in aquaculture (Bentzen et al., 1991, 1994; Wright and Bentzen, 1994; O'Reilly and Wright, 1995).

Microsatellites are a class of nuclear DNA markers that are abundant in all eukaryotic genomes (Tautz, 1989). They consist of repeating sequences of 1 to 5 bases (b) that form arrays less than 300 b in length, and exhibit high levels of co-dominant allelic variation in repeat number (Wright, 1992; Wright and Bentzen, 1994; O'Reilly and Wright, 1995). Polymorphism exhibited by specific microsatellites is readily detected by amplification of the microsatellite through the use of oligonucleotide primers specific to the nonrepetitive regions that flank the repeat array, in combination with the polymerase chain reaction (PCR). Allelic variation is scored by gel electrophoresis of the PCR products, most commonly on denaturing acrylamide gels.

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Microsatellites have begun to be applied in fisheries and aquacultural contexts, and display particular promise in high-resolution population and kinship studies (Bentzen et al., 1991; Wright and Bentzen, 1994; Nielsen et al., 1994; McConnell et al., 1995a, 1995b; O'Reilly and Wright, 1995; Tessier et al., 1995). One advantage of microsatellites as genetic markers stems from the fact that particular microsatellite loci and their flanking sequences are conserved in related species. This means that primers developed for one species can frequently (although not invariably) be used to amplify polymorphic microsatellites in related species. This attribute offers particular benefits for the application of microsatellites in salmonid fishes, for which sequences of more than 50 microsatellite primer pairs have been reported from cloning efforts in several species (see below).

Despite the positive attributes of microsatellites described above, the application of these markers may be limited by operational difficulties including development costs, sample throughput constraints, and allele-scoring difficulties. We address these issues below for Pacific salmon.

As noted above, the availability of numerous primer sequences is an advantage for the application of microsatellites to salmonids. This advantage, however, is offset to some extent by uncertainty about which microsatellite primers are most suitable for particular species and application issues. Here the availability of many primer sequences presents a dilemma of choice. It is relatively costly to purchase and test a large fraction of the available primers before any particular application, but failure to do so can lead to subsequent inefficiencies. A guide summarizing the performance of a wide range of primers would therefore be of general utility.

Sample throughput is another issue that closely affects the cost-benefit ratio of microsatellite application. Most microsatellite analyses have involved typing one locus at a time. However, major gains in throughput as well as savings in consumables and labor are possible through combined analyses of multiple loci, a process known as multiplexing (Urquhart et al., 1995; O'Reilly et al., 1996). Multiplexing is the combination of allele fragments from more than one locus from an individual in a single lane of an electrophoretic gel. This can be achieved by co-amplification of multiple loci in the same PCR, mixing of amplified loci after PCR, or both. Multiplexing using conventional labeling techniques is constrained by the wide allelic range of many salmonid microsatellites (O'Reilly et al., 1996). The use of multicolor fluorescent detection systems can improve throughput by permitting co-amplification of overlapping loci (Ziegle et al., 1992; Paetkau et al., 1995; Urquhart et al., 1995).

Some microsatellite loci, particularly those with dinucleotide repeats, produce ladders of bands following amplification via PCR. These "stutter bands," believed to result from slipped strand mispairing during DNA synthesis, make allele scoring difficult and may lead to misidentification of allelic states using conventional scoring methods (Levinson and Gutman, 1987; Hauge and Litt, 1993; O'Reilly and Wright, 1995). Automated genotyping systems using in-lane sizing standards and fluorescent detection have been shown to increase accurate detection of alleles (Ziegle et al., 1992; Schwengel et al., 1994).

This study has three objectives: First, we assess interspecific priming of various microsatellite primer pairs in Pacific salmon. We do this by completing a broad multispecies screening of 35 salmonid microsatellite primer pairs. These primers, developed from six different salmonid taxa, are tested in five species of *Oncorhynchus* and *Salmo salar*. Second, using the screening results, we demonstrate semiautomated multilocus genotyping by multiplexing six microsatellite loci in sockeye, coho, and chinook salmon and scoring their alleles with a four-color fluorescent detection system. Finally, we provide an indication of the allelic variability of microsatellites in these salmon. The potential application of multilocus microsatellite genotyping to studies of *Oncorhynchus* spp. population structure as well as kinship analysis are discussed here and in a companion paper by Wenburg et al. (this issue).

Results

Microsatellite screening

Table 1 summarizes the PCR annealing temperature, the quality of the PCR product (as described in Figure 1), and the estimated allelic range for each species-locus combination. The quality of some amplifications, particularly those receiving a grade of 3, may be improved by increasing the annealing temperature. The source species for each primer pair was included as a positive control with the exception of the three brown trout-derived loci. The positive control received a score of 3 to 5 in

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Table 1. Salmonid microsatellite screening results.

Microsat. locus	Chinook (T)-(Q)-(S)	Coho (T)-(Q)-(S)	Sockeye (T)-(Q)-(S)	Pink (T)-(Q)-(S)	Chum (T)-(Q)-(S)	Atlantic (T)-(Q)-(S)
Fgt1	(56)-2-3,4	(56)-4-3,4	(56)-2-3	(56)-2-2	(56)-3-4	(56)-4-3,4
Omy77	(50)-2-2	(50)-2-2	(50)-2-1	(50)-5	(50)-5	(50)-2-1
Omy78	(55)-4-1	(55)-4-1	(55)-4-1	(55)-4-1	(55)-4-1	(55)-4-1
Omy87	(55)-4-1,2	(55)-4-2,3	(55)-3-2	(55)-3-1,2	(55)-4-3,4	(55)-4-1,2
Omy207	(56)-1-1	(56)-1-1	(56)-5	(53)-5	(53)-5	(53)-1-1
Omy293	(55)-5	(55)-5	(55)-5	(55)-5	(55)-5	(55)-5
Omy325	(58)-2-1	(58)-2-1,2	(60)-2-2	(55)-2-3	(55)-3-2,3	(58)-2-2,3
Oneμ1	(58)-5	(58)-2-2	(58)-2-1	(58)-2-1	(58)-5	(58)-2-1,2
Oneμ2	(58)-5	(58)-2-2,3	(58)-2-3,4	(57)-5	(57)-2-4	(58)-2-3,4
Oneμ8	(58)-2-2	(55)-2-3	(58)-2-3	(55)-2-4	(55)-2-3	(55)-5
Oneμ10	(57)-5	(57)-5	(57)-2-1,2	(57)-5	(57)-5	(57)-5
Oneμ11	(58)-1-2	(58)-1-2	(58)-1-2	(58)-1-2	(58)-1-2	(58)-1-2
Oneμ14	(58)-2-3	(58)-2-3,4	(58)-2-2	(58)-5	(58)-5	(58)-2-3
Ots1	(50)-1-3	(50)-1-3	(50)-5	(50)-2-3	(50)-2-2	(50)-1-3
Ots2	(48)-2-1	(48)-2-1	(48)-5	(50)-5	(50)-5	(48)-5
Ots3	(50)-2-1	(50)-5	(50)-2-1	(50)-5	(50)-2-1	(50)-1-1
Ots4	(58)-2-2	(54)-2-2	(57)-2-2	(54)-5	(54)-5	(48)-2-1
Ots5	(45)-2-2	(45)-5	(45)-5	(45)-5	(45)-2-2	(45)-5
Ots6	(57)-1-3	(57)-1-3	(57)-5	(57)-5	(57)-5	(57)-5
PuPuPy	(53)-5	(53)-5	(52)-4-5	(53)-5	(53)-5	(53)-5
Sfo8	(60)-2-4	(60)-2-4	(60)-2-4	(55)-2-4	(55)-2-4	(60)-2-3
Sfo12	(50)-5	(50)-5	(50)-5	(50)-5	(50)-5	(50)-5
Sfo18	(52)-5	(52)-5	(52)-5	(52)-5	(52)-5	(52)-5
Sfo23	(52)-5	(52)-5	(52)-5	(52)-5	(52)-5	(52)-5
Ssa4	(57)-2-2	(57)-2-2	(57)-2-2	(57)-3-4	(57)-5	(57)-2-2
Ssa14	(52)-1-2,3	(52)-1-2	(52)-1-2	(52)-1-2	(52)-1-2	(52)-1-2
Ssa85	(58)-2-2	(60)-5	(58)-2-2	(57)-3-3	(57)-3-3,4	(58)-2-1
Ssa171	(56)-1-1	(56)-1-1	(56)-1-1	(57)-5	(57)-5	(56)-1-3,4
Ssa197	(57)-1-3,4	(57)-1-1	(57)-4-1	(57)-1-2	(57)-1-2	(57)-1-2
Ssa202	(58)-5	(58)-5	(58)-5	(58)-5	(58)-5	(58)-5
Ssa289	(46)-5	(46)-5	(46)-5	(46)-5	(46)-5	(46)-5
Ssa293	(53)-2-1	(53)-2-1	(53)-2-1	(53)-2-3	(53)-2-2	(53)-2-1,2
μSat15	(57)-5	(57)-5	(57)-5	(57)-5	(57)-5	(57)-5
μSat60	(60)-2-2	(60)-3-1	(60)-2-2	(57)-2-1	(57)-3-3	(60)-2-2
μSat73	(57)-2-2	(57)-2-2	(57)-3-2	(57)-2-2	(57)-2-1,2	(57)-5

Amplification results are coded as follows: (T) is the PCR annealing temperature; (Q) indicates the quality of the PCR amplification; (S) indicates the approximate size range (bases) of the PCR product. The loci used in multiplex development are shown in bold for chinook, coho, and sockeye. Code for product quality (after Pepin et al., 1995): 1 indicates amplification of one or two bands and no stutter; 2, amplification of one or two bands and some stutter; 3, multiple bands and no smearing; 4, multiple bands and smearing; 5, no amplification at all.
Code for allelic size range: 1 indicates 60–120 b; 2, 120–180 b; 3, 180–240 b; 4, 240–300 b; 5, > 300 b.

eight instances (positive control data for Sfo8, 12, 18, and 23 not shown). This occurred three times in steelhead (see Table 1 in Wenburg et al., this issue) and brook trout (*Salvelinus fontinalis*), and twice in Atlantic salmon. In each case product quality was no better in the other species. As expected, the highest percentage of quality grades 1 and 2 occurred in those species from which some of the microsatellites were developed.

Table 2 summarizes PCR product quality for each species. The loci receiving a quality grade of 1 or 2 are summarized for each species as a percentage of total loci scored. This value ranged from 31% (chum salmon) to 63% (chinook salmon). Only pink and chum salmon had less than 50% of the loci scoring 1 or 2. Between 57% and 74% of the loci given a quality grade of 1 or 2 also had an allelic range of 1 or 2 (60–180 b). In chinook, coho, sockeye,

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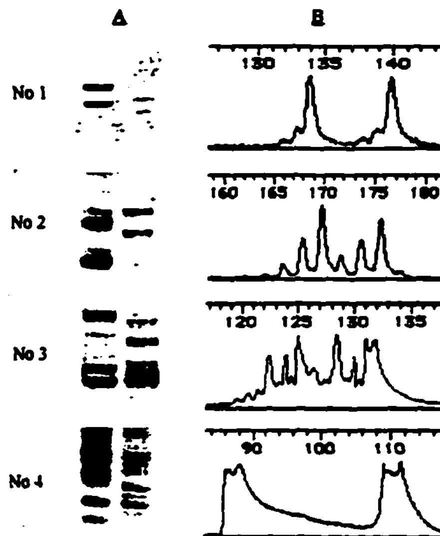

Figure 1. PCR-amplified salmonid microsatellites representing quality grades 1–4. Examples from the Fluorimager 575 and ABI 373A are shown in columns A and B, respectively. Different species-locus combinations were used to depict each quality grade for each detection method. The grading system followed a protocol modified from Pepin et al. (1995) as follows: 1 indicates amplification of one or two bands and no stutter; 2, amplification of one or two bands and some stutter; 3, multiple bands and no smearing; 4, multiple bands and smearing; 5, no amplification.

Table 2. Summary of PCR product quality by species for all microsatellite loci screened.

Species	No. loci screened	Score 1–2	% Score 1–2	Score 3–4	No product
Chinook	35	22	63	2	11
Coho	35	19	54	4	12
Sockeye	35	18	51	5	12
Pink	35	12	34	4	19
Chum	35	11	31	6	18
Atlantic	35	18	51	3	14

chum, and Atlantic salmon, this value was greater than 65%. The small number of loci yielding amplification products greater than 200 b constrained the number of locus combinations for multiplexing.

Multiplex development

Multiplex systems composed of suites of six loci were developed for coho, chinook, and sockeye salmon using 19 of the 35 microsatellite loci (Table 1). In a related project, Wenburg et al. (this issue) developed multiplex systems for cutthroat (*Oncorhynchus clarki clarki*) and steelhead (*Oncorhynchus mykiss*). Our goal of six-locus multiplex suites containing two triplex PCR combinations was based on allele size data from Table 1 that suggested we use each of the three color labels only twice to avoid potential allelic overlap. Further, our initial attempts at co-amplifying microsatellites in sockeye failed frequently when using more than three primer pairs.

Twenty-eight triplex combinations were tested, including 13 in chinook, 9 in coho, and 6 in sockeye. At least two loci co-amplified in 26 of the triplex combinations while three loci co-amplified in 11 combinations. Triplexing was successful in 3 of 13 chinook combinations, 5 of 9 coho combinations, and 3 of 6 sockeye combinations. Two loci, Oneμ10 and Ssa14, did not amplify in the presence of other primer pairs, and one locus, Ssa171, appeared limited to one or two alleles across taxa.

The species-specific triplex sets used in the population survey included a total of nine loci (Table 3). Each triplex reaction was optimized by adjusting individual primer concentrations, annealing temperature, and amount of template DNA. Table 3 shows the reaction conditions and fluorescent label assignments used for each of the six triplex combinations. Primer concentrations ranged from 0.05 to 0.5 μM per reaction. Between 0.5 and 1.0 μl of lysis-prepared DNA was used. The six-locus multiplex suite used to genotype sockeye salmon is depicted in Figure 2. The labeled microsatellite alleles and corresponding electropherograms are shown for two individuals.

Multiple independent screenings of the same individual and locus during multiplex development demonstrated the high resolving power and repeatability of fluorescent-based semiautomated genotyping. Two important observations were made. First, we achieved high allele-scoring precision when PCR conditions were constant for most species-locus combinations. For example, the standard error of estimated allele size at four loci for three independent screenings (PCR and GeneScan) of four heterozygote individuals ranged from 0.03 to 0.06 for coho, chinook, sockeye, and cutthroat (Table 4). Allele size estimates differed by no more than 0.10

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Table 3. Multiplex set composition: fluorescent label assignments,[a] PCR annealing temperature, and primer concentration for each triplex set.

Triplex set	Anneal (°C)	Microsatellite loci and primer concentration (μM)		
		6Fam(blue)	Hex(yellow)	Tet(green)
Chinook A	58		Ssa85(0.30)	Omy325(0.20)/ Oneμ8(0.40)
Chinook B	54	Ots4(0.25)/ Oneμ14(0.60)	Ots1(0.40)	
Coho A	58	Oneμ2(0.25)	Oneμ1(0.50)	Omy325(0.10)
Coho B	54	Oneμ11(0.20)	Ots4(0.45)	Ots1(0.20)
Sockeye A	58	Oneμ14(0.19)	Oneμ1(0.13)	Oneμ11(0.15)
Sockeye B	58	Oneμ2(0.08)	Ssa85(0.15)	Oneμ8(0.16)

[a] 6Fam indicates 6-carboxyfluorescein; Hex, hexachloro-6-carboxyfluorescein; Tet, tetrachloro-6-carboxyfluorescein. The fluorescent labels appear as blue, yellow, and green, respectively, using the ABI filter set B.

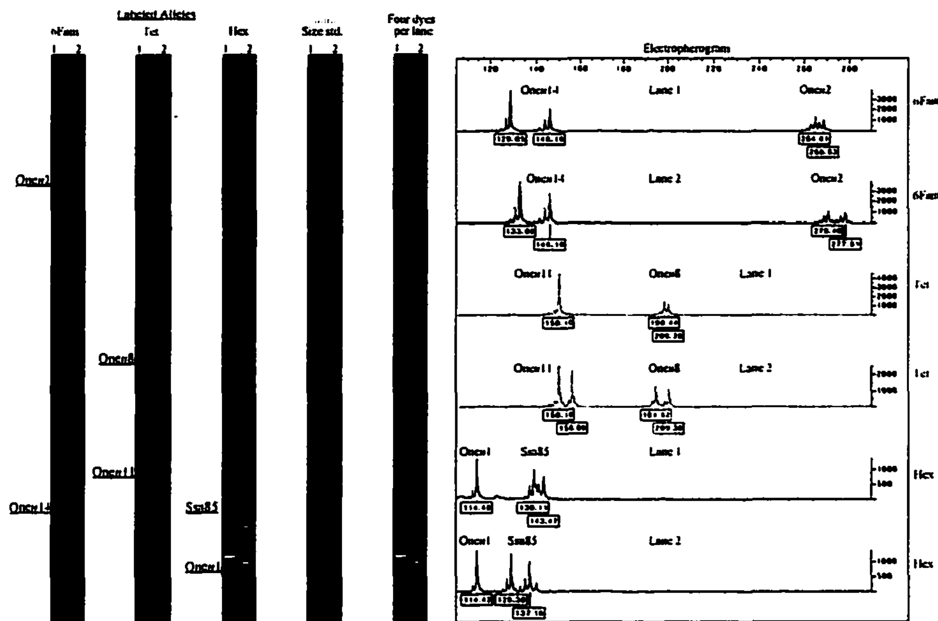

Figure 2. The six-locus multiplex suite used to genotype sockeye salmon. Microsatellites for two individuals were grouped by fluorescent label to illustrate the advantage of four-color fluorescent detection for mutiplexing loci with overlapping alleles. Note: the odd-numbered and even-numbered lanes were loaded approximately five minutes apart to reduce lane assignment errors during data preprocessing. The electropherograms for the same two individuals were also grouped by fluorescent label. The horizontal and vertical axes indicate size (bases) and emission intensity (relative fluorescent units), respectively. Each allele was scored using Genotyper version 1.1 (ABI, 1994) and recorded on the electropherogram. The emission intensity differences between alleles and their "stutter bands" illustrate the level of discrimination afforded by this system and not possible by visual observation of the gel image.

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Table 4. Microsatellite allele scoring variation.

Species	Sample	Locus-allele	Allele-size (b) Run 1	Run 2	Run 3	Avg.	SE
Chinook	C01	Ots4-1	146.35	146.34	146.25	146.31	0.06
		Ots4-2	148.50	148.44	148.42	148.45	0.04
Coho	T03	Oneμ1-1	171.70	171.75	171.67	171.71	0.04
		Oneμ1-2	173.63	173.62	173.56	173.60	0.04
Sockeye	NM01	Oneμ14-1	135.10	135.13	135.20	135.14	0.05
		Oneμ14-2	146.15	146.17	146.20	146.17	0.03
Cutthroat[a]	VI06	Omy77-1	135.11	135.01	135.05	135.06	0.05
		Omy77-2	137.01	136.96	137.02	137.00	0.03

Each run for each species locus combination represents an independent PCR and GeneScan event. Data are from Wenburg et al. (this issue).

b for the three runs, ensuring reproducible scoring of heterozygote individuals with alleles that differ by as little as 2 b (for example, see coho, chinook, and cutthroat in Table 4). By contrast, we found that changing PCR conditions for some species-locus combinations caused a shift of 1 b in scored allele size. For example, reducing the PCR primer concentration from 0.15 to 0.10 μM for Omy325 in coho simplified the product stutter pattern and resulted in a 1-b increase in the most abundant fragment (highest peak on the electropherogram, which is scored as the "true" allele) (Figure 3). Increasing the PCR annealing temperature from 52°C to 57°C for Oneμ11 in sockeye eliminated a single-base stutter and resulted in a 1-b increase in apparent allele size (Figure 3).

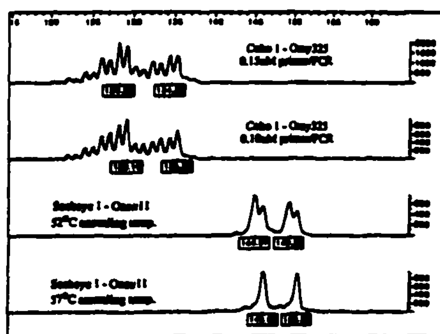

Figure 3. Shifts in allele scoring resulting from changes in PCR conditions. Decreasing PCR primer concentration from 0.15 to 0.1 μM for Omy325 in coho resulted in a 1-b increase (e.g., 128.23 to 129.16) in apparent allele size. Increasing PCR annealing temperature from 52°C to 57°C for Oneμ11 in sockeye eliminated a single-base stutter and resulted in a 1-b increase (e.g., 144.97 to 146.08) in apparent allele size.

Multilocus genotyping

Six-locus multiplex surveys of 50 individuals each of chinook, coho, and sockeye revealed extensive genetic variation (Table 5). The average number of alleles per locus was 13 for chinook, 13 for coho, and 9 for sockeye, although the number of alleles at individual loci was quite variable, ranging from 2 (Oneμ11 in coho and Oneμ1 in sockeye) to 31 (Omy 325 in coho). The allelic size range of individual loci was also quite variable (2–80 b, mean = 30 b). About 75% of the amplified microsatellite products did not exceed 200 b. Of the six loci used in chinook salmon, two with the same fluorescent label (Ots1 and Ssa85) were found to have overlapping allelic ranges. This overlap was due to a rare occurrence of a 147-b allele at the Ots1 locus and was verified by separate amplification and electrophoresis of each locus. The peak profile of each locus appears to produce a unique amplification signature that may be useful for distinguishing rare instances of overlap. For further explanation of signature peak profile, see Figure 2 in Wenburg et al. (this issue)

The relative utility of each locus as a genetic marker for fine-scale genetic studies was assessed by estimating two index parameters. First, PIC provides an index of the informativeness of a locus for use in gene mapping as well as kinship and parentage studies. Values greater than 0.5 are considered highly informative (Botstein et al., 1980). Of the 18 species-locus combinations surveyed, 14 had PIC values greater than 0.5 and 9 had values greater than 0.7. The probability of a match (P_M) (finding two unrelated individuals with the same genotype) was conservatively estimated for each

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Table 5. Allelic variability: number of alleles (A), allele range (R), polymorphic information content (PIC), and match probability (P_M) for the most frequent genotype for each species-locus combination.[a]

Stat.	Omy325	Oneμ1	Oneμ2	Oneμ8	Oneμ11	Oneμ14	Ots1	Ots4	Ssa85	6 Loci
Chinook										
A	10			14		23	6	8	17	13
R	89–125			159–191		183–249	147–195	142–162	118–170	
PIC	0.758			0.873		0.906	0.519	0.681	0.885	0.770
P_M	0.020			0.010		0.010	0.116	0.090	0.020	4.2×10^{-10}
Coho										
A	31	9	28		2		5	4		13
R	92–174	168–184	200–282	140–142		187–199	134–140			
PIC	0.934	0.745	0.824	0.057		0.381	0.333		0.562	
P_M	0.004	0.048	0.004		0.884		0.336	0.384		8.8×10^{-8}
Sockeye										
A		2	11	8	3	9			20	9
R		112–144	282–280	194–214	146–156	129–151			129–187	
PIC		0.136	0.767	0.691	0.511	0.875			0.834	0.602
P_M		0.706	0.032	0.068	0.102	0.078			0.026	3.2×10^{-7}

[a] *N* = 50 for each species.

species-locus combination and each multiplex group using the most common genotype. For most loci P_M values fell below 0.1. Combined P_M values were computed for each multiplex set and were 4.0×10^{-10}, 7.2×10^{-8}, and 3.2×10^{-7} for chinook, coho, and sockeye, respectively.

Table 6 summarizes the observed and expected percentage of heterozygosity for each population-locus combination. Here also, the differences in variability among loci are evident. Heterozygosities ranged from 0 (Oneμ11 in Togiak River coho) to 0.96 (Ssa85 in Dungeness River chinook). No instances of significant departure from Hardy-Weinberg equilibrium were found (initial $\alpha = 0.025$).

Table 7 summarizes the χ^2 pairwise test of independence between populations and allele frequencies. Significant differences were found for all loci except Oneμ1 (sockeye), Oneμ11 and Ots4 (coho), and Ots 1 (chinook) (initial $\alpha = 0.008$). The probability of independence at locus Ots4 (coho) was marginally insignificant ($p = .026$, $\alpha = 0.025$).

Discussion

Interspecific priming of microsatellites

Our screening of microsatellites in Pacific salmon demonstrates that sequence conservation in prim-

Table 6. Observed heterozygosity (H_O), and expected heterozygosity (H_E) for each population-locus combination.

Pop[a]	Stat.[b]	Omy325	Oneμ1	Oneμ2	Oneμ8	Oneμ11	Oneμ14	Ots1	Ots4	Ssa85	Avg./pop
Chin1	H_O	.64			.76		.84	.56	.56	.96	.72
	H_E	.75			.84		.89	.60	.61	.89	.75
Chin2	H_O	.72			.68		.80	.56	.60	.84	.70
	H_E	.86			.82		.81	.61	.77	.82	.75
Coho1	H_O	.92	.76	.80		.00		.08	.36		.49
	H_E	.90	.70	.82		.00		.08	.31		.47
Coho2	H_O	.96	.84	.92		.12		.68	.36		.65
	H_E	.96	.83	.95		.12		.63	.41		.65
Sock1	H_O		.12	.76	.72	.56	.72			.72	.60
	H_E		.12	.80	.75	.51	.76			.73	.61
Sock2	H_O		.20	.68	.64	.36	.52			.84	.54
	H_E		.18	.74	.65	.51	.56			.91	.59

[a] Chin1 indicates Dungeness R.; Chin2, Stolle Meadow, Coho1, Togiak R.; Coho2, Big Beef Ck.; Sock1, Nikolai Ck.; Sock2, Okanagan R.

[b] The difference between H_O and H_E was not significant for any locus-population combination.

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Table 7. Results of the pairwise test of independence between populations and allelic composition.

Populations[a]	Omy325	Oneμ1	Oneμ2	Oneμ8	Oneμ11	Oneμ14	Ots1	Ots4	Ssa85
Chin1 × Chin2									
p	<.001*			<.001*	<.001*	.075	<.001*	<.001*	
SE	.000			.000		.000	.006	.000	.000
Coho1 × Coho2									
p	<.001*	<.001*	<.001*		.246		.001*	.026	
SE	.000	.000	.000		.003		.000	.003	
Sock1 × Sock2									
p		.720	<.001*	<.001*	<.001*	<.001*		<.001*	
SE		.003	.000	.000	.000	.000	.000		.000

The *p* value is the probability of independence between populations and the allele composition. SE is the standard error of the *p* value estimate.
[a] Chin1 indicates Dungeness R.; Chin2, Stolle Meadow; Coho1, Teglek R.; Coho2, Big Beef Cr.; Sock1, Nikolai Cr.; Sock2, Okanagan R. *N* = 25 for each population.
* Probability values judged significant following sequential Bonferroni adjustment (initial α = .0008).

ing regions often permits interspecific exchange of primers. These results are supported by previous findings (e.g., McConnell et al., 1995a, 1995b; Morris et al., 1996), suggesting microsatellite-based genetic studies of Pacific salmon are possible using existing primers. The high cost and time associated with creating microsatellite libraries and designing primers need not be constraints. Further, our data should be helpful in directing researchers to useful species-primer combinations, reducing development costs associated with primer testing.

By exchanging primers across species of salmon, we are assuming they amplify homologous loci. Evidence supporting this assumption has been found in salmonid fishes, mammals, and sea turtles (FitzSimmons et al., 1995; Forbes et al., 1995; Pepin et al., 1995; Morris et al., 1996). However, final verification will require sequencing of the PCR product. This is of particular importance when conducting phylogenetic surveys across taxa (Estoup et al., 1995; Forbes et al., 1995).

In some instances the degree of complementarity between a primer and the microsatellite-flanking sequence may vary among alleles within a species. In extreme cases some alleles will not amplify. The presence of "null" alleles can be inferred through population-level screening and testing for departures from Hardy-Weinberg equilibrium (Callen et al., 1993). Using this approach we did not see evidence of null alleles in the six loci screened in coho, chinook, and sockeye. Likewise, Wenburg et al. (this issue) did not see evidence of null alleles in the loci screened in steelhead and cutthroat. However, in an earlier study, we did observe evidence of one or more null alleles at microsatellite Ssa293 in sockeye (P. Bentzen and J.B. Olsen, unpublished data).

Microsatellite multiplexing and data processing

We have demonstrated the potential for rapid throughput of Pacific salmon microsatellites using one ABI 373A automated sequencer/genescanner and analysis software. Using the six-locus multiplex systems described here and those in Wenburg et al. (this issue), it is possible to process 432 genotypes per day (6 loci × 72 individuals) running two GeneScan gels. We anticipate increasing this rate by multiplexing additional microsatellites. The scarcity of loci greater than 200 to 300 b in our current multiplex systems should allow for added loci. We feel a realistic short-term goal is nine loci (648 genotypes per day), and as more data are gathered on the allelic range of microsatellites in Pacific salmon, it may be possible to exceed 10 loci per lane. In some cases microsatellite-flanking sequences may be used to reposition primers and "customize" loci to maximize vertical gel space on the ABI 373A.

This rate of production results in a large volume of data; hence, efficient analysis requires precision in allele scoring and rapid data processing. We have shown that reproducible allele scoring is possible for dinucleotide microsatellites when heterozygotes may differ by as little as two bases. Using the Genotyper software (ABI, 1994), we have developed species-specific template files that allow rapid allele scoring and data synthesis into formats easily imported by statistical software such as GENEPOP (Raymond and Rousset, 1995).

We found that 1-b shifts in apparent allele size for some microsatellites may occur when changing the PCR temperature profile and reaction mixture. Brownstein et al. (1996) demonstrated that variable adenylation, influenced by the PCR profile and nu-

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cleotide sequence at the 5′ end of the reverse primer, is one mechanism that can control single-base shifts in PCR product size. Brownstein et al. (1996) also showed that the degree of adenylation can be controlled by designing 5′ "tails" for reverse primers and altering the PCR profile. Our results suggest that adherence to a single PCR temperature profile and reaction mixture will help ensure reproducible results when using primers prone to adenylation. However, modification and testing of adenylation-prone salmonid microsatellite primers, following the protocol of Brownstein et al. (1996), will likely result in significant reduction in single-base stutter by promoting or inhibiting adenylation.

Microsatellite-based genetic studies of Pacific salmon

The use of microsatellites for genetic studies of Pacific salmon is in its infancy. Our results provide some of the first indications of the level of polymorphism of these markers in three species of Pacific salmon. The results are encouraging and in general agree with the range of microsatellite variation reported for other fishes and, in particular, Atlantic salmon. The high level of polymorphism found in the multiplexed loci suggests that assessment of fine-scale population structure of Pacific salmon, unresolvable by other genetic markers, may be possible with microsatellites. In general, the PIC values reported here are similar to those reported for microsatellites in mammals (Ostrander et al., 1993; Pepin et al., 1995). Further, the probabilities of match for the individual loci and multiplex suites are similar to those reported by Urquhart et al. (1995) for human microsatellites and O'Reilly et al. (1996) for Atlantic salmon microsatellites. Our data suggest that these markers may be employed when high levels of discrimination are needed, as in kinship studies and parentage analysis.

Experimental Procedures

Microsatellite screening

Microsatellite loci were amplified by the PCR using recently developed salmonid primer pairs (Appendix A). PCRs were performed in a Perkin Elmer 9600 thermocycler. Two individuals were screened from the following species: chinook *(O. tshawytscha)*, coho *(O. kisutch)*, sockeye *(O. nerka)*, pink *(O. gorbuscha)*, chum *(O. keta)*, and Atlantic salmon *(Salmo salar)*. The DNA for microsatellite screening was extracted from a variety of tissues including fin, heart, and liver using the phenol/chloroform method (Hoelzel and Green, 1992). Heart and liver tissue were stored frozen at −70°C, whereas fin tissue was preserved in 100% ethanol. Microsatellite primers were synthesized in the Marine Molecular Biotechnology Laboratory (MMBL) on a Beckman Oligo 1000 DNA synthesizer. PCR was typically carried out in a 10-µl volume (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM $MgCl_2$; 0.8 mM dNTPs; 0.4 units *Taq* polymerase; 0.3 µM primer; and 100–250 ng DNA template). DNA amplifications involved the following profile: one cycle of 94°C (2 min); seven cycles of 94°C (1 min) plus X°C (30 sec) plus 72°C (15 sec); and 18 cycles of 94°C (30 sec) plus X°C (30 sec) plus 72°C (15 sec), where X was an annealing temperature that varied among microsatellites. To account for possible base mismatches during interspecific priming, the annealing temperature was set at 3° to 5°C below the primer melting temperature calculated from the nucleotide composition.

Results of each PCR were assessed using one of two fluorescent detection systems. In most instances we used a Molecular Dynamics FluorImager 575 to detect fluorescently stained microsatellite alleles. Typically, 5 µl of each PCR product and 1 µl of loading buffer (15% w/v ficoll 400, 0.06% w/v bromophenol blue, 0.06% w/v xylene cyanol, 30 mM EDTA) was loaded on a 20-cm, 6% nondenaturing polyacrylamide gel and electrophoresed for approximately two hours at 150 V. At least two lanes of each gel contained 3 µl of Superladder-low 20 × 100 base pair (GenSura Laboratories Inc.) size standard for estimating microsatellite allele length. Following electrophoresis the contents of each gel was stained with a 1:10,000 solution of SYBR Green 1 nucleic acid gel stain (Molecular Probes Inc.) and 1X Tris borate EDTA (TBE) buffer for 30 minutes and scanned on the FluorImager at a PMT voltage of 500–600. An example of a FluorImager gel image used to assess PCR results is shown in Figure 1.

Some microsatellites were detected using a Perkin Elmer–Applied Biosystems Inc. (ABI) 373A DNA Sequencer in GeneScan mode (ABI, 1993). The gel preparation and analysis procedures for this system are discussed below. The ABI 373A produces a linear display (electropherogram) of emission intensity for each of four fluorescent labels (Figures 1 and 2). The PCR results were evaluated by the electropherogram when using the ABI 373A.

Following electrophoresis, two numerical codes

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were recorded for each species-locus combination, indicating the PCR product quality and approximate allele size. A quality code of 1 to 5 was assigned following a protocol modified from Pepin et al. (1995). The quality codes were defined as follows: 1 indicates amplification of one or two bands and no stutter; 2, amplification of one or two bands and some stutter; 3, multiple bands and no smearing; 4, multiple bands and smearing; 5, no amplification at all. Examples of quality codes 1 to 4 are shown in Figure 1 for the two detection systems (note: the examples from each system are represented by different species-locus combinations). The allele sizes were coded as follow: 1 indicates 60–120 b; 2, 120–180 b; 3, 180–240 b; 4, 240–300 b; 5, > 300 b. Loci with alleles near the end of a given range were assigned two size codes.

Multiplex development

Multiplex systems were developed for chinook, coho, and sockeye salmon using the ABI 373A to detect alleles. The ABI 373A uses an argon laser similar to that in the FluorImager but is capable of detecting and distinguishing the emission spectra of four fluorescent DNA labels (Ziegle et al., 1992). In this system, each dye label is depicted as a different color. Up to three loci with overlapping alleles may be multiplexed by labeling a single primer for each locus with a different color. The fourth color is reserved for the internal lane (sizing) standard. One of three colors was assigned to each microsatellite locus based on its observed allelic range previously detected with the FluorImager 575. Labeled primers were synthesized at the MMBL as described above or purchased from Keystone Laboratories (Menlo Park, Calif., U.S.A.).

We attempted co-amplification of various combinations ("triplex sets") of three microsatellite primer pairs for each of the three species. When formulating the species-specific triplex sets, we chose from among those loci initially screened. Only loci with quality codes of 1 or 2 and similar primer annealing temperatures (± 2°C) were considered for grouping. For each species two triplex sets were combined before electrophoresis. Thus, no more than three loci from among any group of six were allowed to share the same allelic range. These criteria reduced the number of possible primer combinations for each species.

Co-amplification of each species-specific triplex was attempted in four individuals. The DNA extraction and PCR were carried out as above except that each PCR cocktail contained a 0.3 μM concentration of each of six primers. The PCR profile described above was used. If the annealing temperature differed for the three primer pairs, then the lowest was used for the initial PCR.

Samples from each PCR were electrophoresed on a 6% denaturing polyacrylamide gel to determine the quality of co-amplification. Approximately 1.0 μl of each PCR was combined with 3.15 μl formamide, 0.60 μl 50 mM EDTA, and 0.25 μl (1.0 fmol) Perkin-Elmer GS350 internal size standard. All samples were denatured at 95°C for approximately three minutes, chilled on ice, and then loaded on the gel. Each gel was run for approximately eight hours at 25 W. Following the gel run, data were analyzed using the local Southern sizing algorithm in the GeneScan 672 analysis software, version 1.1 (ABI, 1993). The electropherogram for each color label was used to determine which of the three loci in each triplex amplified. Those triplexes in which one or more of the loci did not amplify were not tested further. Those groups in which all loci amplified were optimized by adjusting individual primer concentrations to equalize signal intensity as depicted by peak height on the electropherogram (Urquhart et al., 1995; O'Reilly et al., 1996). Two groups of three loci were chosen for each of the three species based on expected levels of polymorphism and quality of co-amplification. Finally, multiple screenings of the same individuals and loci during multiplex development provided a test of the repeatability of this system.

Automated multilocus genotyping with microsatellites

We genotyped two populations from each of the three species using the species-specific multiplex suite of six microsatellites. Twenty-five individuals from each population were screened. The populations included Dungeness River, Puget Sound, Washington (Chinook 1); Stolle Meadow, south fork Salmon River, Idaho (Chinook 2); Togiak River, Western Alaska (Coho 1); Big Beef Creek, Hood Canal, Washington (Coho 2); Nikolai Creek, Kenai Peninsula, Alaska (Sockeye 1); and Okanagan River, Columbia River, Washington (Sockeye 2).

The DNA was extracted using a rapid, simplified cell-lysis protocol modified from Hoelzel and Green (1992). Approximately 5 to 10 mg of tissue was placed in 100 μl of cell lysis buffer consisting of 40 mM Tris-HCl at pH 9.5, 50 mM EDTA, and 0.5% Tween 20. One microliter of proteinase K (10 mg·ml^{-1}) was added to each sample before incubation at 37°C for approximately 12 hours. The sam-

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ples were then heated to 95°C for 15 minutes, centrifuged for 10 minutes at 17,000 × *g*, and frozen at −20°C until needed for PCR. The DNA from coho and Nikolai Creek sockeye populations were obtained from fin tissue preserved in 100% ethanol. The DNA from the Okanagan River population was obtained from liver or heart tissue frozen at −70°C. Chinook DNA prepared using the phenol/chloroform extraction protocol was provided by outside sources.

The PCR profile described above was used for each triplex and population; only the annealing temperature was adjusted. Between 0.5 and 1.0 µl of DNA was used from the stock prepared by the lysis method and 100 to 250 ng was used from phenol/chloroform-extracted DNA. Following PCR, 1 µl from each of the two triplexes was added to a 0.5-ml microtube and combined with 3.15 µl of formamide, 0.60 µl of 50 mM EDTA, and 0.25 µl (1.0 fmol) of Perkin Elmer GS350 Tamra size standard. All samples were denatured at 95°C for approximately three minutes, chilled on ice, and then loaded on the gel. Each individual sample was electrophoresed on the ABI 373A and analyzed automatically with the GeneScan 672 software version 1.1 (ABI, 1993) as described above. Scoring of allele sizes for each locus and tabulation of data for importing into statistical software were performed with Genotyper software, version 1.1 (ABI, 1994)

Statistical analysis

Conformity to Hardy-Weinberg equilibrium was tested to evaluate within-population and between-population genetic variation for each species using the algorithms of Louis and Dempster (1987) and Guo and Thompson (1992). A χ^2 analysis of independence between populations and allelic composition was computed according to Raymond and Rousset (1994). Computations were performed using GENEPOP, version 1.1 (Raymond and Rousset, 1995). Statistical significance levels (α) for the Hardy-Weinberg equilibrium and χ^2 analyses were determined using sequential Bonferroni adjustments for simultaneous tests (Rice, 1989).

Allelic variation and information content of each locus and multiplex suite for each species were measured by combining the data from each population. The number of alleles and allelic range were computed. The polymorphic information content (PIC) was computed for each species-locus combination according to Botstein et al. (1980). Finally, the matching probability (P_M) was computed for each locus and multiplex suite for each species using the most common genotypes (Edwards et al., 1992)

Acknowledgments

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Appendix A

Table A.1 Panel of 35 salmonid microsatellite primers used in this study.

Primer	Primer sequence (F>forward, R>reverse)	Reference	Source species
Fgt1	F>5'-AGA-TTT-ACC-CAG-CCA-GGT-AG, R>5'-CAT-AGT-CTG-AAC-AGG-GAC-AG	Sakamoto et al., 1994	*Oncorhynchus mykiss*
Omy77	F>5'-CGT-TCT-CTA-CTG-AGT-CAT, R>5'-GGG-TCT-TTA-AGG-CTT-CAC-TGC-A	Morris et al., 1996	"
Omy78	F>5'-ACT-CCA-GCA-CAC-CTG-TCT-CC, R>5'-TGT-CTC-AGT-GCT-CTT-TCC-C	Michael O'Connell, personal communication[a]	"
Omy87	F>5'-TCC-TGG-TCT-GGT-GCA-GG, R>5'-ATT-AAC-TCC-GTT-CCA-GCC-G	"	"
Omy207	F>5'-ACC-CTA-GTC-ATT-CAG-TCA-GG, R>5'-GAT-CAC-TGT-GAT-AGA-CAT-CG	"	"
Omy293	F>5'-CAC-AGA-GTG-CGA-TCG-TGG, R>5'-GGT-ACT-AAT-GTT-AAG-CTC-GAG	"	"
Omy325	F>5'-TGT-GAG-ACT-GTC-AGA-TTT-TGC, R>5'-CGG-AGT-CCG-TAT-CCT-TCC-C	"	"
PuPuPy	F>5'-ATG-CAG-CGG-ATG-TAG-GGG-GA, R>5'-TTA-AGT-GAA-AAG-ACG-TAA-GTC	Morris et al., 1966	"
Oneμ1	F>5'-GTC-TTA-CCA-AAT-GTC-TTC-CTC-CT, R>5'-GCC-ATT-TAG-CAT-ACG-ATT-TTA-TC	Scribner et al., 1996	*Oncorhynchus nerka*
Oneμ2	F>5'-GGT-GCC-AAG-GTT-CAG-TTT-ATG-TT, R>5'-CAG-GAA-TTT-ACA-GGA-CCC-AGG-TT	"	"
Oneμ8	F>5'-AAC-ATT-CTG-GGA-TGA-CAG-GGG-TA, R>5'-CTG-TTC-TGC-TCC-AGT-GAA-GTG-GA	"	"
Oneμ10	F>5'-ATG-GGG-AAC-AGA-AGA-GGA-AT, R>5'-CTG-TAG-GTG-TGA-AAT-GTA-TTT-AAA	"	"
Oneμ11	F>5'-GTT-TGG-ATG-ACT-CAG-ATG-GGA-CT, R>5'-TCT-ATC-TTT-CCT-GTC-AAC-TTC-CA	"	"
Oneμ14	F>5'-AGA-AAC-ATG-AGA-ACA-GTC-TAG-GT, R>5'-CCT-TAT-GAG-TTT-GGT-CTC-CAT-GT	"	"
Ots1	F>5'-GGA-AAG-AGC-AGA-TGT-TGT-T, R>5'-TGA-AGC-AGC-AGA-TAA-AGC-A	Dennis Hedgecock personal communication[b]	*Oncorhynchus tshawytscha*
Ots2	F>5'-ACA-CCT-CAC-ACT-TAG-A, R>5'-AAT-ATC-CTT-CAC-ACT-G	"	"

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Table A.1 Continued

Primer	Primer sequence (F>forward, R>reverse)	Reference	Source species
Ots3	F>5'-CAC-ACT-CTT-TCA-GGA-G, R>5'-AGA-ATC-ACA-ATG-GAA-G	[a]	[a]
Ots4	F>5'-GAC-CCA-GAG-GAC-AGC-ACA-A, R>5'-GGA-GGA-CAC-ATT-TCA-GCA-G	[a]	[a]
Ots5	F>5'-ACA-GCA-GTC-TAC-ATT-GAC-C, R>5'-TGT-TCA-TTA-AAA-CCA-AAA-A	[a]	[a]
Ots6	F>5'-TCT-CTT-CCA-GCA-CCA-CAC-A, R>5'-AGA-CAG-TTT-TTC-CAC-ATC-C	[a]	[a]
Sfo8	F>5'-CAA-CGA-GCA-CAG-AAC-AGG, R>5'-CTT-CCC-CTG-GAG-AGG-AAA	Angers et al., 1995	*Salvelinus fontinalis*
Sfo12	F>5'-GGT-TTT-GAA-GAG-TGA-CAG, R>5'-CCC-GTT-TCA-CAA-TCA-GAG	[a]	[a]
Sfo18	F>5'-TGG-TGT-ATC-CTG-CTC-CTG, R>5'-TGG-AAT-GTG-TGT-CTG-TTT-TCT	[a]	[a]
Sfo23	F>5'-GTG-TTC-TTT-TCT-CAG-CCC, R>5'-AAT-GAG-CGT-TAC-GAG-AGG	[a]	[a]
Ssa4	F>5'-ATT-AGG-CAG-CAG-CAG-GCT-GC, R>5'-TGT-TCA-CTC-ACT-GAC-ACG-CG	McConnell et al., 1995a	*Salmo salar*
Ssa14	F>5'-CCT-TTT-GAC-AGA-TTT-AGG-ATT-TC, R>5'-CAA-ACC-AAA-CAT-ACC-TAA-AGC-C	[a]	[a]
Ssa85	F>5'-AGG-TGG-GTC-CTC-CAA-GCT-AC, R>5'-ACC-CGC-TCC-TCA-CTT-AAT-C	O'Reilly et al., 1996	[a]
Ssa171	F>5'-TTA-TTA-TCC-AAA-GGG-GTC-AAA-A, R>5'-GAG-GTC-GCT-GGG-GTT-TAC-TAT	[a]	[a]
Ssa197	F>5'-GGG-TTG-AGT-AGG-GAG-GCT-TG, R>5'-TGG-CAG-GGA-TTT-GAC-ATA-AC	[a]	[a]
Ssa202	F>5'-CTT-GGA-ATA-TCT-AGA-ATA-TGG-C, R>5'-TTC-ATG-TGT-TAA-TGT-TGC-GTG	[a]	[a]
Ssa289	F>5'-CTT-TAC-AAA-TAG-ACA-GAC-T, R>5'-TCA-TAC-AGT-CAC-TAT-CAT-C	McConnell et al., 1995a	[a]
Ssa293	F>5'-TGG-TTA-TTT-GTT-TCC-AGA-G, R>5'-ATC-AGA-TAC-ACA-GAG-ACG-G	[a]	[a]
μSat15	F>5'-TGC-AGG-CAG-ACG-GAT-CAG-GC, R>5'-AAT-CCT-CTA-CGT-AAG-GGA-TTT-GC	Estoup et al., 1993	*Salmo trutta*
μSat60	F>5'-CGG-TGT-GCT-TGT-CAG-GTT-TC, R>5'-GTC-AAG-TCA-GCA-AGC-CTC-AC	[a]	[a]
μSat73	F>5'-CCT-GGA-GAT-CCT-CCA-GCA-GGA, R>5'-CTA-TTC-TGC-TTG-TAA-CTA-GAC-CTA	[a]	[a]

[a] Biology Department, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada.
[b] University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923, U.S.A.

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APPENDIX B: RAW DATA (GENEPOP INPUT FORMAT) FOR CHAPTER 3 ANALYSIS

Bold typeface indicates an individual that was determined to contain putative steelhead alleles as described in the text. Data is in GENEPOP input format. Population codes are as follows: YC = Yellowstone outgroup, McBride Lake; VA = Parker Creek; VC = Covington Creek; VD = Fennel Creek; VF = Double Ditch Creek; VG = Gold Creek; VH = Stavis Creek; VI = Gierin Creek; VJ = Peabody Creek; VK = Goodman Creek; VL = Snahapish River; VM = Oxbow Creek; VN = Wildcat Creek; VQ = Salt Creek.

"StateCutt
Omy77
Oneu11
Ots1
Ots4
Sfo8
Ssa85
pop
YC01 , 3536 4949 7282 2121 2424 3434
YC02 , 3646 4949 7171 2121 2224 3333
YC03 , 3546 4949 8082 2121 2224 3333
YC04 , 3646 4949 7780 2121 2424 3334
YC06 , 3636 4949 8082 2121 2424 3434
YC07 , 3636 4949 8082 2121 2224 3333
YC08 , 3636 4949 7180 2121 2224 3334
YC09 , 3636 4949 8082 2121 2424 3334
YC10 , 4646 4949 7182 2121 2222 3434
YC11 , 3536 4949 8082 2121 2222 3434
YC12 , 3636 4949 8080 2121 2424 3333
YC13 , 3646 4949 8082 2121 2424 3334
YC14 , 3546 4949 7180 2121 2224 3435
YC15 , 3636 4949 7180 2121 2424 3434
YC16 , 3636 4949 8080 2121 2222 3434
YC17 , 3636 4949 7182 2121 2224 3333
YC18 , 3636 4949 7171 2121 2224 3434
YC19 , 3535 4949 7180 2121 2222 3333
YC20 , 3436 4949 7171 2121 2224 3434
YC21 , 3546 4949 8080 2121 2224 3333
YC22 , 3536 4949 8080 2121 2224 3333
YC23 , 3646 4949 7780 2121 2222 3333
YC24 , 3646 4949 8080 2121 2224 3434
YC25 , 3535 0000 0000 2121 0000 3333
pop
95VA01 , 3644 5252 6876 2526 2426 4748
95VA02 , 3636 5252 0000 2525 0000 4747
95VA03 , 3644 5252 7676 2526 2629 4748
95VA04 , 3643 5253 0000 2526 2426 4747
95VA05 , 3236 5253 7678 2526 1624 4347
95VA06 , 3244 5252 5670 2025 2426 4647

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95VA07 , 3636 5252 7076 2525 2626 4747
95VA08 , 3643 5152 5670 2525 0000 4647
95VA09 , 3649 5152 0000 2525 0000 4747
95VA10 , 4449 5252 6876 2526 1828 4848
95VA11 , 3636 5253 7681 2525 1626 4347
95VA12 , 4950 5151 6667 2525 2829 4648
95VA13 , 3643 5353 6666 2526 1826 4047
95VA14 , 3244 5252 0000 2526 2326 4748
95VA15 , 3244 5252 0000 2526 0000 4748
95VA16 , 3636 5253 0000 2525 0000 4347
95VA17 , 3643 5153 0000 2526 1924 4647
95VA18 , 3641 5353 0000 2526 2426 4747
95VA19 , 4449 5152 0000 2525 1919 4748
95VA20 , 3643 5253 0000 2025 1616 4647
95VA21 , 3244 5252 0000 2425 0000 4748
95VA22 , 3643 5252 5656 2026 2426 4647
95VA23 , 3244 5252 0000 2526 0000 4748
95VA24 , 3636 5252 0000 2526 0000 0000
95VA25 , 3644 5152 7676 2525 2629 4347
95VA26 , 3344 5252 7676 2526 2426 4748
95VA28 , 3643 5353 0000 2526 0000 4647
95VA29 , 3236 5252 0000 2526 0000 4748
95VA30 , 3644 5252 0000 2526 0000 4748
95VA31 , 3644 5152 0000 2525 0000 4347
95VA32 , 3244 5252 0000 2526 2429 4748
95VA33 , 3644 5252 0000 2526 0000 4748
95VA34 , 3644 5252 0000 2526 0000 4748
95VA35 , 3344 5252 0000 2526 0000 4748
95VA36 , 3644 5252 0000 2526 2640 4748
95VA37 , 3244 5252 0000 2526 0000 4748
95VA38 , 3344 5252 0000 2526 0000 4748
95VA40 , 3336 5252 0000 2525 0000 4347
95VA41 , 4244 5151 7682 2626 1819 4350
95VA42 , 3643 5152 0000 2526 0000 4047
95VA43 , 3644 5252 0000 2526 0000 4748
95VA44 , 3038 5252 0000 0000 0000 4243
95VA45 , 4344 5252 7070 2025 1616 4647
95VA46 , 3643 5252 0000 2525 2626 4747
95VA47 , 3643 5252 0000 2025 1624 4647
95VA48 , 4349 5152 0000 2526 1925 4748
95VA49 , 0000 5252 0000 2526 0000 4748
95VA50 , 3636 0000 6475 2526 2425 4848
pop
95VC02 , 3237 5153 7373 2525 2424 4654
95VC03 , 3232 5152 7173 2025 1818 4646
95VC04 , 3748 5152 7373 2025 1830 4545
95VC05 , 4144 5151 7173 2525 2430 3346
95VC06 , 4343 5253 7171 2525 2425 4654
95VC07 , 3341 5151 6573 2026 2429 4546
95VC08 , 3333 5153 7171 2525 3030 3855
95VC09 , 0000 0000 1871 2526 0000 0000
95VC10 , 4344 5151 7171 2629 1824 3852

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95VC11 , 3237 5151 6573 2025 2430 3354
95VC12 , 3244 5151 7373 2128 1829 3345
95VC13 , 4448 5151 7173 2627 2426 3854
95VC14 , 3237 5151 6573 2025 1824 3346
95VC15 , 3640 5253 0000 2030 0000 4348
95VC16 , 4043 5153 2473 2525 2664 3865
95VC17 , 4343 4951 1824 2030 6061 3041
95VC18 , 4156 5151 6677 2020 1626 0000
95VC19 , 4043 4951 1859 0000 2461 2745
95VC20 , 4141 0000 6685 2525 2631 0000
95VC21 , 3636 5153 7379 2025 2431 4145
95VC22 , 3650 5253 7070 2525 2828 4848
95VC23 , 4450 5252 7071 2525 2529 4565
95VC24 , 4150 5252 7378 2525 1924 3847
95VC25 , 3642 5153 1873 2526 3640 2747
95VC26 , 5050 5152 5473 2025 2427 4348
95VC27 , 4041 5152 0000 2525 2632 4048
95VC28 , 4041 5151 7373 2525 2731 4365
95VC29 , 4040 5152 5473 2025 1926 4547
95VC30 , 4343 5151 1858 2025 5966 3940
95VC31 , 4041 5151 3573 2627 2030 0000
95VC32 , 4141 5153 5473 2525 3031 4547
95VC33 , 3638 5152 6673 2525 2627 4545
95VC34 , 4044 5151 5378 2525 3030 4347
95VC35 , 3849 5151 7477 2025 2528 0000
95VC36 , 3849 5151 6873 2525 3031 4348
95VC37 , 4141 5152 7073 2626 1931 4547
95VC38 , 4141 5153 5479 2526 3131 4347
95VC39 , 3744 4949 1818 2020 5861 2740
95VC40 , 4043 5151 2473 2025 2664 3843
95VC41 , 4044 5353 6173 2525 2425 5165
95VC42 , 3644 5353 6680 2525 1819 4345
95VC43 , 4040 0000 6161 2525 1826 4345
95VC44 , 3535 4951 1818 2526 3840 0000
95VC45 , 3636 5152 7373 2025 2428 4343
95VC46 , 4848 4951 1873 2121 1866 4046
95VC47 , 0000 5152 6673 2525 2430 4654
95VC48 , 3742 4949 1858 0000 5866 3256
95VC49 , 4848 0000 1866 2525 1861 3946
95VC50 , 3244 5152 7373 2525 2430 4855
pop
95VD01 , 3843 5151 6684 2525 1520 4952
95VD02 , 4146 5151 5454 2025 0000 4548
95VD03 , 4444 5151 5454 2525 1820 4352
95VD04 , 3841 5151 5454 2525 0000 4549
95VD05 , 3839 5151 0000 2525 0000 4553
95VD06 , 3638 5151 6666 2526 1826 5252
95VD07 , 4350 5151 0000 2025 0000 4343
95VD09 , 3839 5151 0000 2525 0000 4345
95VD10 , 4343 5151 0000 2424 0000 4143
95VD11 , 3638 5151 0000 2526 0000 4141
95VD12 , 4343 5151 6670 2026 0000 4852

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95VD13 , 4143 5151 0000 2525 0000 4048
95VD14 , 3238 5151 5066 2026 0000 3845
95VD15 , 3843 5151 0000 2525 0000 4552
95VD16 , 3943 5151 0000 2525 0000 4348
95VD17 , 3844 5151 5454 2525 0000 4352
95VD18 , 4141 5151 0000 2525 0000 4141
95VD19 , 4444 5151 0000 2525 0000 5253
95VD20 , 3638 5151 5454 2526 1826 4143
95VD21 , 3643 5151 0000 2525 0000 4545
95VD22 , 3637 5151 6679 2526 1626 3843
95VD23 , 3941 5151 0000 2626 0000 4141
95VD24 , 0000 5151 0000 2627 0000 0000
95VD25 , 3238 5151 5070 2525 2026 4549
95VD26 , 3841 5151 7079 2626 2426 3845
95VD27 , 3641 5151 6970 2526 1826 5253
95VD28 , 4143 5151 0000 2526 0000 4045
95VD29 , 3844 5151 0000 2525 1820 5253
95VD30 , 3243 5151 7684 2525 1827 3852
95VD31 , 3643 5151 5466 2525 1520 4952
95VD32 , 4043 5151 7979 2626 1826 3845
95VD33 , 3243 5151 5079 2526 1826 4949
95VD34 , 3243 5151 0000 2525 0000 6060
95VD35 , 4143 5151 6666 2525 2426 4152
95VD36 , 4142 5151 0000 2526 0000 4545
95VD37 , 3643 5151 5454 2526 2426 4141
95VD38 , 4143 5151 0000 2525 0000 5260
95VD39 , 0000 5151 0000 2525 0000 3840
95VD40 , 3644 5151 6670 2525 2526 4041
95VD41 , 4150 5151 6679 2525 1620 4952
95VD42 , 4144 5151 0000 2526 1824 4153
95VD43 , 3641 5151 5454 2525 2424 4852
pop
95VF01 , 3243 5153 0000 2026 2529 3946
95VF02 , 4143 5353 0000 2525 2525 4656
95VF03 , 4343 5152 0000 1925 1829 3940
95VF04 , 3744 4953 0000 2028 0000 3039
95VF05 , 4142 5151 6673 2025 2829 4657
95VF06 , 4142 5151 6673 2025 2829 4657
95VF07 , 4344 5152 0000 2020 0000 3946
95VF08 , 4142 5153 0000 2025 0000 3344
95VF09 , 4344 5151 0000 2025 2329 4663
95VF10 , 4344 5153 0000 2020 0000 3946
95VF11 , 4143 5153 0000 2525 0000 4646
95VF12 , 0000 5153 0000 2020 0000 0000
95VF13 , 4143 5153 0000 2026 0000 3939
95VF14 , 4143 5152 0000 2020 1627 4456
95VF15 , 4449 5353 0000 2525 1730 4657
95VF16 , 4344 5152 0000 2525 0000 4646
95VF17 , 4144 5151 0000 2025 0000 3957
95VF18 , 3249 5153 0000 2020 0000 4453
95VF19 , 3249 5153 0000 2025 0000 4453
95VF20 , 3638 5153 1867 2025 2639 4046

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95VF21 , 4344 5152 0000 2020 2530 3946
95VF22 , 4344 5152 0000 2025 0000 3946
95VF23 , 4343 5151 6666 2025 2425 4456
95VF24 , 3741 5152 0000 2025 0000 4652
95VF25 , 4143 5152 6772 2025 0000 3356
95VF26 , 4143 5153 0000 2025 0000 4646
95VF27 , 4344 5153 0000 2025 0000 4646
95VF28 , 3244 5153 6667 2026 1830 3939
95VF29 , 4141 5151 7073 2626 2427 3757
95VF30 , 4142 5151 6666 2525 2529 4457
95VF31 , 3244 5153 6772 2526 2430 3946
95VF32 , 4449 5153 6667 2526 1618 3946
95VF33 , 3943 5151 0000 2025 0000 3752
95VF34 , 4244 5151 6682 2525 2424 4054
95VF35 , 4244 5151 0000 2525 1919 4054
95VF36 , 4244 5151 0000 2526 2430 4054
95VF37 , 3132 5353 6667 2525 1830 4657
95VF38 , 4143 5151 6774 2025 2528 3757
95VF39 , 4348 5151 6673 2025 3039 3946
95VF40 , 4149 5153 6682 2525 1628 4857
95VF41 , 3643 5153 6673 2026 1631 4452
95VF42 , 4344 5151 6767 2525 2425 4957
95VF43 , 3646 5154 1818 2026 2429 3746
95VF44 , 4343 5153 0000 2026 0000 3946
95VF45 , 4243 5153 6770 2025 2829 3944
95VF46 , 4243 5152 7381 2025 2424 3944
95VF47 , 4343 5151 6666 2025 2628 4446
95VF48 , 3243 5353 6681 2026 1824 3939
95VF49 , 4249 5153 7382 2525 2525 4457
95VF50 , 4143 5151 0000 2525 2525 3744
pop
95VG01 , 3638 0000 6666 0000 2424 4043
95VG02 , 3643 5151 7179 2525 2428 4956
95VG03 , 4243 5151 7583 2525 2530 4646
95VG04 , 3643 5151 7579 2025 2225 4352
95VG05 , 3644 5151 7087 2526 1825 5661
95VG06 , 4346 5152 6667 2525 2425 4046
95VG07 , 4446 5151 7172 2526 1826 4352
95VG08 , 3643 5151 6673 2526 2526 4052
95VG09 , 4349 5151 6681 2525 1828 4648
95VG10 , 4346 0000 6781 0000 1839 0000
95VG11 , 3643 5151 6681 2525 2424 4348
95VG12 , 4343 5152 7181 2525 1825 4667
95VG13 , 3636 5151 7071 2526 1828 5261
95VG14 , 3642 5153 7072 2020 2525 4956
95VG15 , 3743 5151 0000 2025 0000 4652
95VG16 , 3640 5353 0000 2525 0000 4646
95VG17 , 4446 5153 6671 2525 1926 5252
95VG18 , 3641 5151 7179 2020 1825 4352
95VG19 , 4043 5151 7281 2021 1838 4146
95VG20 , 3643 5151 6681 2222 1825 4661
95VG21 , 4349 5151 7071 2222 1825 4852

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95VG22 , 4343 5151 7272 2525 2538 6164
95VG23 , 3636 5252 6672 2224 1828 4043
95VG24 , 4344 5151 6770 2025 1826 4649
95VG25 , 3843 5151 7272 2525 2438 4552
95VG26 , 3642 5152 6771 2222 2538 4656
95VG27 , 4041 5151 7273 1522 1824 4652
95VG28 , 3843 5151 6672 2020 1824 0000
95VG29 , 3643 5151 7072 1919 1924 0000
95VG30 , 0000 0000 7282 2222 1824 0000
95VG31 , 3642 5151 6783 2525 2528 4674
95VG32 , 4346 5151 6464 2025 1818 4652
95VG33 , 3641 5153 7079 2126 2424 4348
95VG34 , 4346 5151 7172 2020 2526 5252
95VG35 , 4043 5153 7281 2525 1838 4646
95VG36 , 3644 5353 6666 1622 2425 4852
95VG37 , 4343 5151 7273 2020 2425 4561
95VG38 , 3644 5151 6670 2222 1939 4652
95VG39 , 4249 5151 8181 2020 1539 4346
95VG40 , 4244 5151 7273 2525 2528 4646
95VG41 , 3843 5252 6679 2525 2424 3143
95VG42 , 3646 5151 7072 2525 1828 5261
95VG43 , 3838 5253 7279 2525 2628 3156
95VG44 , 3643 5151 7070 2525 1939 4661
95VG45 , 3642 5151 6672 1919 1828 5252
95VG46 , 3642 5151 6770 2525 1828 4661
95VG47 , 4346 5151 7070 1919 1928 4661
95VG48 , 4446 5151 7070 2223 1939 4652
95VG49 , 3943 5151 6667 2525 2425 4652
95VG50 , 4043 5153 0000 2525 0000 4065
95VG51 , 4041 5152 7081 2525 2525 4552
95VG52 , 3840 5153 7173 2526 1828 4550
95VG53 , 3843 5151 7072 2526 2530 3852
95VG54 , 4151 5152 6671 2525 2425 5665
95VG55 , 4144 5151 7375 2526 1618 4040
pop
95VH01 , 3941 5353 7172 2526 1828 4657
95VH02 , 3636 5151 6780 2526 1618 3852
95VH03 , 3651 5153 0000 2025 1825 4049
95VH04 , 3641 5153 7075 2025 2525 4343
95VH05 , 4848 4951 1818 2526 5365 4052
95VH06 , 4242 4949 1818 2025 5365 3840
95VH07 , 3641 5152 7682 2525 2525 4346
95VH08 , 3643 4951 6670 2025 1824 4145
95VH09 , 4848 4951 1854 2025 1864 2752
95VH10 , 3743 5151 7079 2525 2528 4874
95VH11 , 3651 5153 0000 2529 1830 4345
95VH12 , 4344 5151 7079 2729 1518 4074
95VH13 , 3743 5151 7081 2025 2830 5274
95VH14 , 3640 4949 7079 2020 1824 4345
95VH15 , 4343 5152 6775 2528 1518 4346
95VH16 , 4040 5151 7171 2025 2424 5256
95VH17 , 3839 5153 7070 2525 1525 4052

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95VH18 , 4343 5353 7173 2525 2830 4246
95VH19 , 4046 5153 7179 2525 1826 4045
95VH20 , 3643 5151 7070 2525 1830 4346
95VH21 , 4049 5152 6870 2025 2530 4774
95VH22 , 4343 5151 7070 2525 1830 4046
95VH23 , 3641 5152 6773 2020 1525 4345
95VH24 , 4251 5353 7071 2025 1825 4552
95VH25 , 4351 5153 7375 2525 1818 4043
95VH26 , 4143 5253 7073 2526 2538 4345
95VH27 , 4046 5151 7079 2525 1624 4348
95VH28 , 3536 5151 5470 2026 2427 4042
95VH29 , 3243 5152 0000 2025 1930 4352
95VH30 , 3637 5153 7082 2025 1530 4052
95VH31 , 4242 4951 1854 2025 1864 4052
95VH32 , 4242 4949 1854 2526 5365 2738
95VH33 , 3641 5151 6773 2025 2525 4346
95VH34 , 4242 4949 1818 2626 1864 2738
95VH35 , 4242 4951 1818 2525 1853 4052
95VH36 , 3743 5151 7082 2025 2528 4874
95VH37 , 4248 4949 1854 2529 6465 2738
95VH38 , 3638 5353 7172 2728 2428 4247
95VH39 , 3643 5153 7079 2025 1530 4052
95VH40 , 3643 5353 7281 2728 2628 4552
95VH41 , 4143 5153 7981 2529 2428 4343
95VH42 , 4242 4949 1854 2025 1864 2738
95VH43 , 4848 4949 1818 2728 1853 3840
95VH44 , 4248 4951 1818 2525 1864 2752
95VH45 , 3741 0000 7079 2025 2324 6065
95VH46 , 4143 5151 7079 2125 1818 5252
95VH47 , 3643 5353 7575 2526 1818 4041
95VH48 , 4248 4951 1854 2728 5264 4052
95VH49 , 4248 4949 7377 2729 0000 3840
95VH50 , 4043 5152 7579 2528 1525 4545
95VH51 , 3237 5151 7373 2526 2424 3346
95VH52 , 3237 5153 7373 0000 2424 4654
95VH53 , 3232 5152 7173 2020 1818 4646
95VH54 , 4248 4949 0000 0000 1864 3840
95VH55 , 4043 5152 7579 2729 1525 4545
95VH56 , 3337 5151 7373 2026 2424 3346
pop
95VI01 , 0000 5153 5778 2526 2426 4849
95VI02 , 4141 5353 5778 2125 2426 3856
95VI03 , 4141 5153 7581 2526 1930 4856
95VI04 , 4344 4951 6473 2526 2630 4851
95VI05 , 3642 5153 1973 2025 1927 4856
95VI06 , 4141 5151 5778 2526 1530 3849
95VI07 , 0000 5153 6469 2026 1526 4851
95VI08 , 4446 4953 6773 2526 1524 4251
95VI09 , 4141 5353 5778 2025 2630 4856
95VI10 , 4143 5153 7078 2626 3030 4256
95VI11 , 4142 4951 6770 2526 2426 4548
95VI12 , 4141 5153 5778 2526 1530 3849

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95VI13 , 4141 4953 2475 2526 1928 4856
95VI14 , 4141 5153 1878 2025 2630 3856
95VI15 , 4244 5153 7378 2526 1530 3842
95VI16 , 4142 4951 7073 2125 2630 4548
95VI17 , 4141 5153 5781 2526 2630 4956
95VI18 , 3141 4953 5781 2626 2630 4256
95VI19 , 4446 5153 6770 2525 1930 3851
95VI20 , 3642 5153 7078 2025 2627 4856
95VI21 , 4949 5353 6769 2125 2628 4856
95VI22 , 4142 4953 6773 2525 1819 4856
95VI23 , 4243 5153 7181 2121 1519 4343
95VI24 , 4646 5153 7072 2025 2830 4856
95VI25 , 4246 5353 5964 0000 1928 3856
95VI26 , 4142 4953 7373 0000 1819 4256
95VI27 , 4246 5151 7173 2020 2428 4848
95VI28 , 4146 5353 7073 1523 2633 4356
95VI29 , 3642 5153 7273 0000 2428 4856
95VI30 , 4042 5353 7373 0000 2830 4345
95VI31 , 4246 5353 7073 2020 1826 3843
95VI32 , 4146 5353 6981 1523 2633 4851
95VI33 , 4649 5153 6467 1522 2628 4356
95VI34 , 4049 5151 6781 1523 1928 4348
95VI35 , 3641 5353 6781 0000 2828 4351
95VI36 , 4149 5151 6467 0000 1827 5656
95VI37 , 4143 5353 6467 0000 1526 5656
95VI38 , 4146 4953 7173 1524 2633 4348
95VI39 , 4042 5353 7173 1522 1818 4548
95VI40 , 3844 5153 6767 0000 1527 4551
95VI41 , 4646 5153 7381 2121 1929 0000
95VI42 , 4244 5153 5981 1523 1928 4548
95VI43 , 4246 5151 7281 1523 1826 5656
95VI44 , 3842 5153 7172 0000 2428 4856
95VI45 , 4248 5153 6767 1524 1919 4848
95VI46 , 4146 5151 6973 0000 2630 4245
95VI47 , 3642 5153 6471 0000 2628 4856
95VI48 , 3844 5153 5471 1524 1519 4343
95VI49 , 3642 5153 7273 0000 2626 4856
95VI50 , 4243 5153 6771 1524 1526 4956
95VI51 , 4244 5153 6781 0000 2628 5656
95VI52 , 3642 5153 7172 1523 2626 4956
95VI53 , 4243 5353 5481 1515 1930 4343
95VI54 , 3642 5353 7172 1515 2426 4856
95VI55 , 4143 5153 6481 0000 2828 4956
95VI56 , 4344 5153 7073 1521 1526 3838
95VI57 , 4949 5151 6767 2222 2626 4856
95VI58 , 4142 5153 6773 1523 2426 5156
95VI59 , 4243 5353 6781 2121 1927 4351
95VI60 , 4646 5153 6471 2126 1926 4356
pop
95VJ01 , 3636 0000 0000 2025 0000 0000
95VJ02 , 3641 5151 7070 2020 2627 4949
95VJ03 , 3644 5151 7071 2020 2425 4349

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95VJ04 , 3644 5353 7070 2020 2126 0000
95VJ05 , 3649 5151 7071 2020 1924 4949
95VJ06 , 4144 5151 0000 2025 1925 0000
95VJ07 , 4449 5151 7073 2025 2627 4351
95VJ08 , 4444 5151 0000 2025 2626 0000
95VJ09 , 3649 5151 7373 2021 2424 4949
95VJ10 , 3644 5153 0000 2020 0000 0000
95VJ11 , 0000 5151 7073 0000 1825 4549
95VJ12 , 0000 5151 0000 2020 0000 4349
95VJ14 , 0000 5153 7070 0000 1924 4949
95VJ15 , 3636 5153 7373 2025 2425 4351
95VJ16 , 0000 5151 0000 0000 1924 4349
95VJ17 , 3641 5151 7171 2020 1927 4749
95VJ18 , 3636 5151 0000 2020 1924 4949
95VJ19 , 3244 5151 7073 2020 1927 4349
95VJ20 , 3244 5353 6869 2020 1925 4343
95VJ21 , 4444 5151 7073 2020 1927 4349
95VJ22 , 4444 5151 7173 2025 1927 4343
95VJ23 , 4444 5151 7071 2025 0000 4343
95VJ24 , 4449 5153 7071 2021 1927 4349
95VJ25 , 4444 5151 7070 2021 1925 4349
95VJ26 , 3236 5153 7171 2020 2626 4343
95VJ27 , 4449 5153 7071 2020 1925 4343
95VJ28 , 3641 5151 7373 2020 1924 4349
95VJ29 , 4444 5151 7070 2025 1926 4351
95VJ30 , 4448 5151 7070 2020 2425 4343
95VJ31 , 3249 5153 7073 2020 2530 4343
95VJ32 , 3644 5153 7070 2021 1925 4343
95VJ33 , 4444 5151 7173 2020 1926 4343
95VJ34 , 4444 5151 7070 2025 1925 4343
95VJ35 , 3644 5151 7071 2021 1927 4343
95VJ36 , 4849 5151 7073 2020 2426 4349
95VJ37 , 4449 5353 7070 2020 1925 4349
95VJ38 , 4444 4951 7073 2020 2526 4349
95VJ39 , 3644 5153 7071 2025 1927 4347
95VJ40 , 3244 5153 7171 2020 1927 4349
95VJ41 , 4449 5153 7171 2020 1925 4343
95VJ42 , 3244 5151 6673 2125 1819 4549
95VJ43 , 4444 5151 7070 2020 2727 4949
95VJ44 , 4449 5153 6670 2020 1925 4343
95VJ45 , 4144 5151 7073 2020 2627 4349
95VJ46 , 3236 5153 7070 2525 1926 4351
95VJ47 , 3244 5151 7071 2021 0000 0000
95VJ48 , 4444 5151 7071 2020 2627 4347
95VJ49 , 4444 5153 6569 2020 2527 4343
95VJ50 , 4449 5153 7173 2020 1924 4349
95VJ51 , 3249 4951 6871 2020 2530 4343
95VJ52 , 4449 5153 7171 2021 2627 4349
95VJ54 , 4444 5153 7071 2021 2527 4349
95VJ55 , 3644 5151 6868 2021 2525 4349
95VJ56 , 3248 5153 7071 2025 1925 4347
95VJ57 , 3644 5153 7071 2021 1927 4343

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95VJ58 , 4444 5151 7073 2025 1925 4343
95VJ59 , 4444 5151 6666 2025 2527 4343
pop
95VK01 , 3838 5151 7475 2128 2739 5262
95VK02 , 3248 5152 5076 2025 2626 3371
95VK03 , 4346 5152 6972 2526 2526 3455
95VK04 , 0000 5153 7385 0000 1527 4959
95VK05 , 4346 5151 6972 2626 2526 3462
95VK06 , 3232 5151 6876 2020 2626 5275
95VK07 , 4956 4953 6285 2026 1528 3849
95VK08 , 3856 5153 7285 2020 2640 4949
95VK09 , 4848 5353 7685 2326 2740 4956
95VK10 , 4646 5151 5075 2028 1926 6162
95VK11 , 4346 5151 6983 2026 2526 3462
95VK12 , 4648 5153 7376 2526 1524 4959
95VK13 , 3146 5151 8385 2626 2626 3462
95VK14 , 0000 5151 5061 2020 2238 7173
95VK15 , 0000 5151 8585 0000 2238 7173
95VK16 , 3232 5152 5075 2528 1639 6262
95VK17 , 0000 5151 1859 0000 0000 3041
95VK18 , 3243 5151 8585 2020 2226 7173
95VK19 , 4848 5353 7685 2326 2740 4956
95VK20 , 3243 5151 0000 2025 1619 4275
95VK21 , 4346 5151 6983 2026 2641 4462
95VK22 , 3232 5151 6972 2026 2626 4462
95VK23 , 4343 5153 8385 2026 1926 3438
95VK24 , 3243 5151 0000 2026 0000 4444
95VK25 , 3131 5152 8385 2025 1626 5252
95VK26 , 3844 4951 6262 2025 1927 4951
95VK27 , 4343 5151 7285 2026 1941 3434
95VK28 , 4346 5151 0000 2626 0000 3462
95VK29 , 3246 0000 5050 2025 1626 6162
95VK30 , 3434 4951 2359 1927 5456 2740
95VK31 , 3738 4951 6272 2025 1924 4949
95VK32 , 3232 5151 6876 2025 1619 4276
95VK33 , 4656 4953 6285 2026 1528 4949
95VK34 , 4848 5153 7685 2025 1524 3859
pop
95VL01 , 3232 5252 0000 2026 0000 4242
95VL02 , 3237 5151 7783 2026 2636 3549
95VL03 , 3737 4952 7583 2025 2536 7277
95VL04 , 3246 4952 7783 2026 2536 4977
95VL05 , 3737 4951 7782 2026 2536 3549
95VL06 , 3242 4949 7275 2025 3838 4272
95VL07 , 4249 5252 0000 2525 0000 4961
95VL08 , 0000 5253 7272 2526 2222 6173
95VL09 , 4646 5152 7583 2027 2626 6177
95VL10 , 3737 5152 7782 2026 2526 4977
95VL11 , 4646 4952 6282 2027 2225 6177
95VL12 , 4646 4952 7582 2020 2626 4278
95VL13 , 3242 5252 6275 2526 3838 4662
95VL14 , 4343 5252 6262 2526 0000 4261

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95VL15 , 3242 5252 7275 2526 3838 4662
95VL16 , 3737 5151 7583 2025 2636 3572
95VL17 , 3246 5252 6262 2025 0000 6274
95VL18 , 0000 5151 6262 2020 2526 3542
95VL19 , 3249 4951 7576 2020 2438 4249
95VL20 , 4646 4951 7782 2026 2626 3549
95VL21 , 4249 5153 5282 2526 1924 3549
95VL22 , 4646 4951 6283 2027 2226 3561
95VL23 , 4246 5151 0000 2026 2538 4649
95VL24 , 4246 5152 0000 2525 0000 4949
95VL25 , 0000 5152 0000 2127 2538 4144
95VL26 , 4349 5252 7373 2525 2441 5972
95VL27 , 3737 5151 7782 2026 2636 3549
95VL28 , 0000 5152 0000 2525 2224 0000
95VL29 , 3232 5252 6262 2025 2626 4962
95VL30 , 4249 5151 5275 2025 2641 4971
95VL31 , 4949 4949 0000 2025 0000 4249
95VL32 , 3232 5152 6262 2026 2441 7071
95VL33 , 4949 4949 7576 0000 2438 4249
95VL34 , 4249 5152 6262 2526 2226 4973
95VL35 , 3246 5152 7582 2026 2526 4977
95VL36 , 3246 4952 7783 2026 2626 4977
95VL37 , 3737 5151 7783 2025 2526 3572
95VL38 , 4646 5151 7582 2020 2225 3561
95VL39 , 3137 4951 7583 2025 2636 3572
95VL40 , 3137 4951 7783 0000 2536 3572
95VL41 , 4646 4951 7783 2525 2441 3561
95VL42 , 3137 4951 7783 2025 2626 3572
95VL43 , 3636 5152 6290 2026 2626 4449
95VL44 , 4343 5151 7275 2025 2224 4144
95VL45 , 3737 5151 7582 2020 2225 3542
95VL46 , 3146 5252 6262 0000 2226 4473
95VL47 , 4646 5151 7783 2025 2636 3571
95VL48 , 3143 4951 6262 2026 3838 4949
95VL49 , 0000 0000 6282 2025 2226 0000
95VL50 , 3643 5252 7175 0000 0000 0000
95VL51 , 4242 5151 6275 2526 2238 3562
95VL52 , 3737 4952 7582 2121 2526 4277
95VL53 , 4646 5151 5275 2020 2226 4949
95VL54 , 3131 5252 7576 2025 2838 4671
95VL55 , 3146 4951 7783 2025 2526 3572
95VL56 , 3137 5151 7583 0000 2536 3572
pop
95VM01 , 4652 5151 6571 2328 2426 3862
95VM02 , 4244 5153 6667 2020 2424 4563
95VM03 , 4246 5152 6466 2528 2633 3535
95VM04 , 3139 5151 6683 2028 1825 3538
95VM05 , 3941 5151 6666 2023 1828 4560
95VM06 , 4242 5151 7575 2020 2424 4769
95VM07 , 3244 5153 5667 2326 1526 4545
95VM08 , 3946 5151 0000 2028 0000 4860
95VM09 , 4251 5153 5675 2026 2628 3547

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95VM10 , 4244 5153 6667 2020 2426 4563
95VM11 , 3242 5151 7575 2020 2026 4563
95VM12 , 4244 5151 6875 2025 2426 4170
95VM13 , 0000 5151 6681 2023 2441 4860
95VM14 , 4042 5151 7575 2020 2424 6369
95VM15 , 3342 5151 6667 2020 2024 4247
95VM16 , 3144 5151 5666 2020 2526 3848
95VM17 , 4251 5152 5675 2028 2628 4761
95VM18 , 4258 5152 7375 2025 1826 4774
95VM20 , 3346 5151 6667 2028 1826 3848
95VM21 , 3941 5151 6666 2028 1828 3848
95VM22 , 3241 5152 6877 0000 2626 3360
95VM23 , 4158 5151 6875 2025 2426 4774
95VM24 , 3241 0000 0000 2023 2526 3848
95VM25 , 4258 5151 6675 2528 2430 4761
95VM26 , 3244 5153 6774 2025 2626 4647
pop
95VN01 , 4343 5151 6771 2525 1515 4647
95VN02 , 0000 5151 0000 0000 1515 4647
95VN03 , 4949 5151 7376 2025 2328 4777
95VN04 , 0000 5151 6269 0000 2424 4462
95VN05 , 4349 5151 6973 2525 2425 4762
95VN06 , 4349 5151 7679 2628 1538 6263
95VN07 , 4355 5151 6975 2125 2223 4860
95VN08 , 4349 5151 5675 2828 2526 4462
95VN09 , 4243 5151 6269 2525 2426 4760
95VN10 , 4243 5151 7375 2525 1524 4462
95VN11 , 3743 5151 5675 2025 2425 3544
95VN12 , 4949 5151 0000 2528 0000 4444
95VN13 , 4949 5151 6969 2028 1526 4447
95VN14 , 4246 5151 6979 2020 1520 4757
95VN15 , 4349 5151 7576 2026 1524 4554
95VN17 , 4949 5151 7575 2025 2326 4447
95VN18 , 4449 5151 5656 2021 2324 4446
95VN19 , 4449 5151 7173 2021 2426 4447
95VN20 , 4649 5151 6476 2528 2838 5463
95VN21 , 4449 5151 6971 2525 1520 0000
95VN22 , 4949 5151 6476 2628 2328 5463
95VN23 , 4646 5151 7575 2121 1922 0000
95VN24 , 4249 5151 6969 2021 2324 4447
95VN25 , 4356 5151 5675 2020 2425 3544
pop
95VQ01 , 3232 5151 0000 2525 1830 4245
95VQ02 , 3241 5151 6573 2028 2930 3346
95VQ03 , 3043 5152 0000 1925 2429 3041
95VQ04 , 3741 0000 0000 2025 2629 0000
95VQ05 , 4444 5253 7173 2026 2530 4652
95VQ06 , 3741 0000 6566 0000 1824 0000
95VQ07 , 3333 5151 7373 0000 2430 4654
95VQ08 , 3237 5151 7173 2525 3030 4646
95VQ09 , 3241 5151 0000 2125 2630 3345
95VQ10 , 3237 5153 6565 0000 1824 4648

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95VQ11 , 3348 5151 0000 2028 1824 4646
95VQ12 , 3232 5153 6567 2025 2424 0000
95VQ13 , 3348 5151 7373 2525 2430 4545
95VQ14 , 3232 5151 6573 2025 2629 4648
95VQ15 , 3741 5152 7171 2025 1830 5454
95VQ16 , 3244 5152 7373 2020 2429 4546
95VQ17 , 3232 4951 0000 0000 1866 0000
95VQ18 , 3637 5253 7373 2125 2430 4546
95VQ19 , 3337 5153 0000 2525 2430 0000
95VQ20 , 4444 5151 6573 0000 2429 4546
95VQ21 , 3248 5151 7173 2121 2929 3345
95VQ22 , 4448 5153 6573 2525 2930 4548
95VQ23 , 0000 0000 0000 2525 1824 4546
95VQ24 , 4141 5153 5454 2025 1829 4347
95VQ25 , 4141 5151 0000 2023 2629 0000
95VQ26 , 3232 0000 6670 2028 2425 0000
95VQ27 , 4148 0000 7173 2525 1830 4546
95VQ28 , 3648 5151 0000 0000 1824 3348
95VQ29 , 3244 5151 0000 2525 2629 4546
95VQ30 , 3741 5151 0000 1928 1826 3848
95VQ31 , 3333 5151 0000 2020 0000 0000
95VQ32 , 3840 5152 0000 2525 1919 4547
95VQ33 , 0000 5252 6678 2526 2426 4051
95VQ34 , 4250 5153 6173 2021 2527 6565
95VQ35 , 3136 5152 5473 2525 2431 4748
95VQ36 , 3841 5151 6779 0000 1925 4547
95VQ37 , 4350 5151 5454 2028 3264 3843
95VQ38 , 4050 5152 7373 2525 1530 3845
95VQ39 , 3641 5153 0000 2125 1925 4365
95VQ40 , 4244 5151 5858 1919 5967 2940
95VQ41 , 3849 5152 0000 0000 3031 3845
95VQ42 , 4043 5151 5454 2028 3268 3843
95VQ43 , 3042 4951 5858 2828 2560 3041
95VQ44 , 3750 5153 0000 2027 2664 0000
95VQ45 , 3737 5151 0000 0000 2731 4345
95VQ46 , 4243 5153 0000 2830 2436 4040
95VQ47 , 3644 5252 0000 0000 2630 4345
95VQ48 , 3641 5153 6681 0000 2426 4848
95VQ49 , 3636 5353 0000 2020 2629 4565
95VQ50 , 3840 5152 7373 2525 1920 4751
95VQ51 , 3850 5253 0000 2525 3131 4045
95VQ52 , 4150 0000 7385 2025 2040 2743
95VQ53 , 0000 5153 0000 2025 2424 4343
95VQ54 , 3337 4949 0000 1927 0000 3856"

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APPENDIX C: RAW DATA (GENEPOP INPUT FORMAT) FOR CHAPTER 4 ANALYSIS

Bold typeface indicates an individual that was determined to contain putative steelhead alleles as described in the text. Data is in GENEPOP input format. Population codes are as follows: TH=Thorndyke; TB=Tarboo; F=Fulton; C=Courtney; B=Bear; BM=Big Mission; ST=Stavis; SB=Seabeck; BB=Big Beef; LA=Little Anderson.

"Omy77
Oneu11
Ots1
Ots4
Sfo8
Ssa85
Oneu2
Ots100
Ssa14
Ots103
pop
TH01 , 0000 0000 0000 0000 0000 0000 2527 3033 1820 1010
TH02 , 4343 5151 0000 2025 1830 0000 1825 2430 1923 1010
TH03 , 3643 5353 7073 2526 1824 4652 1922 3033 1820 1010
TH04 , 4143 5353 7073 2025 1830 4650 2325 3033 1919 1010
TH05 , 3643 5353 7073 2026 1828 4675 2227 0000 1919 1010
TH06 , 4143 5253 5470 2025 2630 5075 2525 2937 1919 1010
TH07 , 3338 5353 7073 2020 1839 4648 2325 2137 1819 1010
TH08 , 4244 5151 7373 2525 1624 4548 1922 2930 1820 1010
TH09 , 3642 5353 7070 2025 2526 4546 2525 2937 1924 1010
TH10 , 3338 5153 7379 2525 1539 4546 2527 2020 1919 1010
TH11 , 3643 5153 5470 2025 1825 4546 1825 3038 1923 1010
TH12 , 3643 5153 5470 2025 1826 4652 0000 0000 0000 1010
TH13 , 3643 5153 7073 2021 1828 4575 1919 3037 1819 1010
TH14 , 3338 5153 7079 2025 1825 4548 2425 3337 1923 1010
TH15 , 4143 5151 7073 2025 3030 4652 2425 3337 1923 1010
TH16 , 4143 5151 7073 2025 3030 4652 2327 3738 1923 1010
TH17 , 3843 5153 7071 2021 2830 4575 1622 1414 1923 1010
TH18 , 3840 5152 7382 2626 2528 4546 2325 3037 1920 1011
TH19 , 4446 5153 5487 2025 2526 4546 1825 3037 1919 1010
TH20 , 3636 5152 5467 2025 1630 4357 1623 3738 1919 1010
TH21 , 4040 5353 7073 2025 1839 4849 1619 3738 1519 1010
TH22 , 3644 5353 6773 2025 1618 4348 2325 3738 1923 1010
TH23 , 3336 5353 6673 2026 2430 4675 2226 2238 0000 0910
TH24 , 3644 5353 6770 2525 1828 4348 2226 2238 1923 1010
TH25 , 4651 5151 7379 2020 3030 5075 1825 3738 2323 1010
TH26 , 3843 5353 6673 2026 2430 4675 1825 3738 1923 1010
TH27 , 3336 5353 7073 2026 2430 4675 0000 0000 0000 0000
TH28 , 3342 5153 7079 2525 1830 4952 2123 3037 1923 1010
TH29 , 3651 5253 7073 2025 1925 4548 2325 3038 1519 1010
TH30 , 3338 5353 7073 2020 2526 4648 2525 2930 1919 1010

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TH31 , 4044 5153 5470 2025 1828 4243 1819 3737 1519 1010
TH32 , 3644 5353 5470 2025 1618 4348 1619 3037 1519 1010
TH33 , 3338 5153 7073 2125 1828 4552 2327 3037 1923 1010
TH34 , 3336 5153 7071 2125 2830 4552 1827 2437 1923 1010
TH35 , 3636 5151 5473 2025 2330 4549 2323 3037 1919 1010
TH36 , 3343 5151 5470 2025 2630 4674 2527 3038 1819 1010
TH37 , 4043 5151 5470 2525 1818 4252 1819 3038 1819 1010
TH38 , 4143 5151 5470 2025 1826 4646 1925 3738 1920 1010
TH39 , 3643 5151 6773 2025 1618 4243 1925 3038 1920 1010
TH41 , 3636 5353 7073 2025 1824 4849 1925 3738 1920 1010
TH42 , 3844 5454 6671 2227 2830 4774 1625 2238 1919 1010
TH43 , 4650 5353 5473 2020 1628 4348 2325 2430 2323 1010
TH44 , 3338 5353 7073 2526 2430 4652 2226 2238 1919 1010
TH45 , 3351 5153 7379 2525 1825 4546 1825 3038 1919 1010
TH46 , 3642 5353 0000 2020 1839 4648 2525 2929 1924 1010
TH47 , 3336 5353 0000 2526 2430 4652 2525 2929 1919 1010
TH48 , 3233 5151 7179 2525 2525 4552 2325 2228 1919 1010
TH49 , 3341 0000 0000 2025 1826 4646 2325 2228 1819 1010
TH50 , 0000 0000 0000 0000 0000 0000 0000 3038 1920 1010
Pop
TB01 , 4242 5151 7171 2026 2425 4173 2525 2530 1923 1010
TB02 , 3846 5152 5454 2026 2526 4048 2525 1025 1923 1010
TB03 , 4243 5151 5454 2525 2425 5252 1930 3033 1819 1010
TB04 , 4346 5353 6679 2020 1825 4252 1625 1428 1919 1010
TB05 , 4043 5151 6666 2020 1525 5257 1820 2429 1919 1010
TB06 , 3844 5151 5473 2025 1826 3845 2227 1437 1919 1010
TB07 , 4349 5152 7173 2026 2426 4149 2525 3032 1919 1010
TB08 , 3248 5153 5470 2020 1825 4552 1821 0000 1519 1010
TB09 , 4144 5253 7171 2026 1818 5252 1820 3237 1919 1010
TB10 , 3340 5151 6670 2025 1818 5252 1623 3237 1923 1010
TB11 , 4243 5153 7081 2026 2425 4849 2527 2832 1818 1010
TB12 , 4649 5151 7172 2025 1825 4348 2025 2930 1919 1010
TB13 , 4449 5151 0000 2526 2828 4143 2525 3033 1919 1010
TB14 , 4243 5153 6666 2525 1525 4852 1927 1232 1919 1010
TB15 , 3846 5253 5466 2025 2425 4052 1825 1025 1923 0910
TB16 , 4249 5153 6681 2525 1824 5052 1818 2537 2123 1010
TB17 , 4242 5152 6671 2026 2426 4148 2525 2530 1919 1010
TB18 , 4449 5151 6681 2026 2830 4148 1818 3237 1919 1010
TB19 , 3343 5151 7381 2828 2123 4853 2323 3637 1921 0911
TB20 , 0000 5151 5471 2026 2830 4148 1825 3337 1819 1010
TB21 , 4249 5151 5454 2025 2430 5252 1822 3337 1919 0910
TB22 , 4349 5153 8081 2525 1818 4352 2325 2828 1923 1010
TB23 , 4449 5151 7179 2020 1818 4346 2227 1237 1919 1010
TB24 , 3846 5253 5471 2026 2525 4048 1825 1025 1923 1010
TB25 , 3849 5151 6673 2025 2426 5052 2525 2837 2323 0910
TB26 , 3536 5152 6673 2025 1825 4865 2325 3037 1921 1010
TB27 , 4349 5253 0000 2525 1626 4848 1927 2828 1923 1010
TB28 , 4951 0000 5472 2025 2530 4352 1825 3033 1819 1010
TB29 , 4452 5151 5466 2525 1525 5252 2527 3237 1919 1010
TB30 , 4244 5151 5454 2020 2830 4852 1818 3337 1819 1010
TB31 , 4449 5153 6666 2020 1838 4352 2425 2828 1919 1010
TB32 , 0000 5153 6671 2025 1825 4357 0000 3237 1919 1010

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TB33 , 4449 5151 6673 2025 1838 5257 2525 1433 0000 1010
TB34 , 4449 5151 7073 2025 2425 4352 2525 3738 1923 1010
TB35 , 4449 5153 6771 2025 1828 5274 2526 2228 1824 1010
TB36 , 4344 5151 2481 2025 2425 4249 2225 1230 1922 1010
pop
F01 , 3358 5151 8282 2525 2931 4750 1519 0000 1919 0910
F02 , 4358 5151 7682 2525 1531 5359 1923 2323 1919 1010
F03 , 4344 5151 7182 2525 2930 4242 1820 0000 1923 1010
F04 , 4044 5151 0000 2021 2228 4144 0000 0000 1919 0000
F05 , 4258 5151 7682 2525 1531 5059 1420 2323 1819 1011
F06 , 3333 5151 6977 2025 1522 4667 1414 2323 1919 1010
F07 , 4043 5151 8182 1920 1528 4144 1115 3638 1919 1011
F08 , 4344 5153 8383 2525 1526 4141 1419 2323 1923 0910
F09 , 3344 0000 0000 0000 0000 0000 0000 0000 0000 0000
F10 , 4343 5153 7171 2121 1532 5961 1419 3337 1921 1011
F11 , 4044 5153 7273 2025 2731 4459 1119 3333 1919 1011
F12 , 4344 5153 7983 2025 2130 4862 2331 3636 1919 1111
F13 , 4044 5151 8182 2021 2628 4144 1120 3838 1919 0911
F14 , 3344 5151 7272 2020 2628 4450 1118 3438 1919 1010
F15 , 3344 5151 0000 2525 1518 4059 0000 0000 1921 0000
F16 , 4358 5151 5673 2525 1532 5059 1420 2338 1819 1011
F51 , 3344 0000 0000 0000 0000 0000 1519 2350 1919 0910
F52 , 3333 5353 0000 2525 1919 4242 0000 0000 0000 0000
F53 , 3333 5353 0000 2025 1527 4759 0000 0000 1919 0000
pop
C01 , 0000 0000 0000 0000 0000 0000 1725 3437 1819 1010
C02 , 3544 5151 7887 2025 1725 4070 2527 3737 1919 1010
C03 , 4243 5151 6978 2121 1625 4950 1926 3036 1922 1010
C04 , 3544 5151 7887 2025 1725 4070 2527 3737 1919 1010
C05 , 3743 5353 6667 2529 1858 3946 1830 2830 1921 1010
C06 , 4344 5153 7379 2025 1725 4974 1820 2838 1924 1010
C07 , 4249 4952 6670 2028 3959 3670 2330 1942 1922 1016
C08 , 3643 5151 6773 2025 2529 4356 2125 2237 1923 1010
C09 , 4040 5151 6666 2025 2424 4145 1823 0000 1922 1010
C10 , 4246 5151 6772 2526 2425 4046 2527 2232 1919 1010
C11 , 3544 5153 5467 2025 2525 4070 1827 3737 1719 1010
C12 , 4244 5153 7887 2025 2539 5270 1718 3737 1719 1010
C13 , 4143 5151 7379 0000 2628 4346 2225 2231 1823 1010
C14 , 4243 5151 6687 2525 2839 5252 1723 3737 1919 1010
C15 , 3543 5153 7071 2125 1818 4351 1419 3738 1919 1010
C16 , 3333 5153 6670 2525 1624 4952 2127 3737 1919 1010
C17 , 4142 4951 7382 2025 2959 4243 2225 2430 2122 1016
C18 , 3542 0000 7087 2525 1725 4043 1927 3838 1919 1010
C19 , 3642 5151 6787 2525 1825 4043 2327 0000 0000 1010
C20 , 4042 5153 5467 2025 2539 5270 1725 3737 1722 1010
C21 , 4043 5151 6671 2020 2426 4570 1822 3237 1919 1010
C22 , 4044 5153 6771 2025 1524 3843 1923 2537 1819 1010
C23 , 3943 5153 6772 2025 2525 5256 1921 0000 1922 1010
C24 , 0000 0000 0000 2525 0000 0000 2225 3237 1919 1010
C25 , 0000 0000 0000 2025 0000 0000 1723 3237 1819 1010
C26 , 3540 5151 0000 2025 2525 5270 1718 0000 1922 1010
C27 , 3543 5152 6666 2525 2526 4352 2727 3132 1919 1011

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C28 , 3843 5153 5475 2020 1526 4652 2526 2837 1819 1010
C29 , 3636 5151 7181 2525 2839 3864 2222 1919 1919 1010
pop
B01 , 0000 5151 6667 0000 1525 4874 0000 0000 0000 0000
B02 , 4040 5153 7378 2025 2428 4356 0000 3737 1921 1010
B03 , 4044 5151 6771 2025 1525 5252 2325 3738 1923 1011
B04 , 3942 5151 6679 2024 2425 7074 0000 2232 0000 1010
B05 , 3543 5151 6666 0000 2527 4564 0000 0000 0000 0000
B06 , 4149 5153 6671 2020 2525 4647 0000 1414 1919 1010
B07 , 3343 5152 6670 2025 2525 4056 1823 3737 1921 1010
B08 , 4243 5152 7070 2525 1618 5252 2527 2830 1919 1010
B09 , 3844 5151 6770 2020 2533 3856 2326 2838 1919 0000
B10 , 0000 5253 6266 2125 1825 4852 0000 0000 1921 1010
B11 , 4043 5153 5481 2025 2525 3856 2325 3737 1921 1010
B12 , 4249 5152 6670 2026 1824 4952 0000 0000 0000 1010
B13 , 4344 5153 6970 2025 2528 4952 2526 3037 1919 1010
B14 , 4044 5151 6873 2020 2426 4852 1719 0000 1919 1010
B15 , 4044 5153 6671 2026 1839 3152 2527 2837 1919 1010
B16 , 4040 5253 7378 2025 1828 4956 1723 1437 2122 1010
B17 , 4043 5153 5470 2020 2525 4043 1825 3637 1921 1010
B18 , 3340 5253 5468 2020 2425 4056 1923 2237 1919 1010
B19 , 3842 5152 5467 2025 2426 4374 0000 2937 0000 1010
B20 , 4244 5153 6669 2020 2428 3152 1827 2228 1919 1010
B21 , 4246 5153 6283 2024 1824 4661 2327 3037 1718 1010
B22 , 4444 5151 7071 2025 2525 4052 2525 2837 1919 1010
B23 , 4042 5153 7887 2025 2525 3152 1725 0000 1919 1010
B24 , 4243 5153 6669 2026 1617 3850 1922 2837 1922 1010
B25 , 4249 5151 7072 2020 2228 4252 2727 2837 1919 1010
B26 , 4246 5151 6271 2024 1824 4061 1818 3037 1919 1010
B27 , 4243 5151 7070 2020 1618 3138 1825 2830 1919 1010
B28 , 4343 5152 6670 2025 2425 5256 1923 2237 1819 1010
B29 , 3343 5253 7079 2026 2525 3152 2527 2836 1819 1010
B30 , 5151 5151 7173 2526 2425 3843 1825 2728 1923 1010
B31 , 3840 5153 7174 2026 1830 5256 2327 2337 1921 1010
B32 , 4044 5151 6771 2025 1525 5252 2325 3838 1923 1011
B33 , 4951 5152 7173 2020 2526 4748 1723 1442 1919 1010
pop
BM01 , 0000 0000 0000 2525 0000 0000 0000 0000 0000 0000
BM02 , 4043 5151 7181 2626 2528 4348 0000 0000 1922 0000
BM03 , 3536 5151 6671 2525 2628 4545 2525 3737 1923 1010
BM04 , 3343 5152 6671 2226 2638 4546 2528 0000 1919 1010
BM05 , 4343 5151 6781 2525 1838 4748 2222 0000 1519 1010
BM06 , 0000 0000 0000 2525 0000 0000 0000 0000 0000 0000
BM07 , 3749 5152 7175 2727 1838 4040 0000 0000 0000 0000
BM08 , 3642 5151 6671 2525 1938 4345 2325 2938 1919 1010
BM09 , 3538 5151 0000 2025 1826 4345 0000 0000 0000 0000
BM10 , 4042 5152 7475 2627 1825 4040 2230 1437 1922 1010
BM11 , 4344 5152 6671 2026 1828 4052 2325 1222 1922 0910
BM12 , 3636 5153 6770 2525 2225 4352 2223 3030 1919 1010
BM13 , 4343 5153 6670 2126 1818 4952 2525 2238 1819 1010
BM14 , 3640 5151 1818 2025 2526 4647 2023 0000 1919 1010
BM15 , 3637 5153 7075 2525 1825 4549 2225 3037 1819 1010

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BM16 , 3539 5151 7070 2525 1825 3856 0000 0000 0000 0000
BM17 , 4349 5151 7581 2425 2225 3852 1927 1422 1919 1010
BM18 , 3644 5151 5466 0000 2425 4852 1825 1222 1919 1010
BM19 , 0000 0000 0000 2025 0000 4352 0000 0000 0000 0000
BM20 , 3539 5151 7073 2525 1833 4856 1826 0000 1919 1010
BM21 , 3540 5151 1818 2528 2538 2948 2548 1028 2323 1016
BM22 , 3551 5153 6771 2025 2533 4152 0000 0000 0000 0000
BM23 , 3643 5353 0000 0000 0000 4549 0000 0000 0000 0000
BM24 , 3537 5151 0000 2121 0000 4548 2227 0000 1919 1010
BM25 , 4344 0000 0000 1924 0000 0000 2222 0000 1919 1010
BM26 , 3536 0000 6565 2025 2434 4251 2227 0000 1919 0000
BM28 , 0000 0000 0000 2126 0000 0000 1823 1414 1919 1010
BM29 , 3540 5151 6675 2227 1628 4552 2027 2222 1919 1010
BM30 , 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000
BM31 , 3944 5153 0000 2626 1818 4348 0000 0000 0000 0000
BM32 , 3543 5353 6770 2025 2526 5252 2325 2238 1919 0910
BM33 , 3636 5151 7070 0000 2533 4565 0000 0000 0000 0000
BM34 , 4243 5153 7579 0000 1838 4552 0000 0000 0000 0000
BM35 , 3643 5151 7070 2626 2528 5256 2325 3838 1919 0910
BM36 , 3540 5151 7171 2222 1826 4043 0000 0000 1919 1010
BM37 , 3543 5151 7181 0000 1828 4852 0000 0000 0000 0000
BM38 , 4043 5252 7373 2020 1838 4770 1825 0000 1919 1010
BM39 , 3636 5152 6670 2525 1825 4570 1820 1222 1519 1010
BM40 , 3841 4951 0000 2529 3639 4040 2743 1428 1919 1010
BM41 , 3538 5153 7071 0000 1833 3348 0000 0000 0000 0000
BM42 , 3943 5153 6771 2025 2525 5252 2325 2230 1922 0910
BM43 , 4444 5252 7070 2525 1825 5252 2225 3039 1921 1010
BM44 , 4344 5152 7275 2525 1825 5256 0000 0000 0000 0000
BM45 , 3542 5153 6770 2525 1826 3856 0000 0000 1919 0000
BM46 , 3343 5152 7072 2025 2538 4346 2325 0000 1919 1010
BM47 , 3640 5151 5466 2025 2525 4664 2223 3030 1919 1010
BM48 , 3644 5153 6671 2025 2426 4345 2227 3737 1519 1010
BM50 , 3643 5152 7070 2020 1825 4952 1818 3738 1819 1010
pop
ST01 , 3640 5253 0000 2020 0000 4046 0000 0000 1919 0000
ST02 , 3349 0000 7073 2025 1818 4657 2727 0000 1923 1010
ST03 , 4243 5151 6773 2525 1625 5274 1825 3737 1923 1010
ST04 , 3651 5151 6773 2025 2530 5256 2124 1414 1823 1010
ST05 , 4151 5151 7070 2025 1624 4545 1927 2222 1819 1010
ST06 , 3846 5151 7576 2525 2430 4557 1820 2424 1823 1010
ST07 , 4251 5151 7272 2021 2425 4648 1923 2732 1919 1010
ST08 , 3643 5252 6770 2020 1818 4052 2326 2832 1919 1010
ST09 , 3640 5153 7178 2025 1627 4046 1722 2837 1919 1011
ST10 , 4243 5153 7082 2526 2425 4247 2328 2237 1819 1010
ST11 , 4043 5151 7073 2025 1828 4548 1825 1417 1519 1010
ST12 , 3743 5153 7173 2025 1830 4055 2427 1437 1823 1010
ST13 , 5151 5153 6770 2020 1925 4650 2223 2437 1919 0910
ST14 , 4151 5153 7082 2526 1819 4346 2426 3236 1823 1010
ST15 , 3646 4949 7981 2025 1830 4052 2526 2537 1923 1010
ST16 , 3940 5153 7375 2025 2758 4248 2223 1224 1921 1011
ST17 , 4141 4951 1873 2027 1572 0000 1616 1031 1921 1010
ST18 , 3641 5153 7879 2626 2538 4042 1823 3637 1919 1010

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ST19 , 3949 5253 7173 2025 1825 4850 1720 2737 1919 1010
ST20 , 4040 5151 7073 2526 2527 4146 2327 2437 1819 1011
ST21 , 3643 5153 7073 2126 1516 4965 1623 2737 1819 1010
ST22 , 3642 5151 5479 2526 2530 4143 2326 0000 1823 1010
ST23 , 4246 5151 7173 2525 2425 4952 2525 1437 1819 1010
ST24 , 4040 5151 7182 2025 1515 4550 2425 3737 1923 1010
ST25 , 4044 5153 6773 2025 3033 4046 1823 2437 1919 1010
ST26 , 3743 5151 7879 2026 1825 4952 1823 1438 1819 0910
ST27 , 3646 5153 7282 2026 2424 4546 2125 2428 1919 1010
ST28 , 3640 5152 6770 2525 1818 4346 2525 3037 1919 1010
ST29 , 4143 5151 7071 2526 1519 3848 1827 3237 1919 1010
ST30 , 3640 5153 7070 2525 1825 3846 2225 1437 1919 1011
ST31 , 4042 5151 7179 0000 2430 3852 2527 3738 1519 0910
ST32 , 4142 4951 7173 2025 1827 4646 2325 1424 1919 1111
ST33 , 3740 5353 5478 2526 1825 3849 2325 2222 1919 1010
ST34 , 4041 5153 6770 2626 2430 3852 1619 2537 1923 1010
ST35 , 4243 5151 6771 0000 2528 0000 2425 1430 1819 1010
ST36 , 3741 4953 7273 2126 1825 5050 2225 1414 1919 1010
ST37 , 4244 5151 7071 2525 2529 4648 2122 1424 1919 1010
ST38 , 3851 5153 6767 2526 1825 4648 2323 2432 1819 0910
ST39 , 3349 5253 7173 2525 1818 4557 2727 2837 1923 1010
ST40 , 4243 5153 6670 2025 1824 4345 2324 2437 0000 1010
ST41 , 3842 5151 7375 2525 2425 4545 2324 2538 1919 1010
ST42 , 4044 5152 7582 2526 1825 4750 2528 2237 1919 1010
ST43 , 0000 4951 8181 2525 1618 0000 2225 2532 1923 1010
ST44 , 0000 5151 7070 2526 1830 0000 1725 1437 1919 1010
ST45 , 3641 5152 6678 2020 1827 4648 1721 1437 1919 1010
ST46 , 0000 5152 7171 2525 1625 4857 1727 2737 1919 1010
ST47 , 3651 5151 7072 2525 1825 4248 1627 2424 1919 1010
ST48 , 4040 5153 7078 2526 1827 4652 2327 2437 1819 1011
ST49 , 3846 5151 6781 2526 3030 0000 1625 3237 1923 1010
ST50 , 4046 5153 7379 2626 1825 4550 2527 1422 1823 1010
pop
SB01 , 0000 0000 0000 0000 0000 0000 2225 2828 1819 1010
SB02 , 3644 5151 6672 2525 2530 4148 2525 2738 1919 1010
SB03 , 4044 5353 6666 2025 2525 4048 2525 1438 1919 1010
SB04 , 4042 5151 5471 2025 2528 4152 2326 1414 1519 1010
SB05 , 4648 5153 7171 0000 1825 4050 2325 1422 1919 1010
SB06 , 3838 5151 5466 2525 2526 4850 1925 1222 1919 1010
SB07 , 3746 4951 1818 1925 2640 3950 2235 1414 1926 1015
SB08 , 4243 5151 7273 2525 1828 5061 1725 1424 1819 1011
SB09 , 3343 5253 7378 2025 1638 4750 1823 2238 1923 1010
SB10 , 3840 5153 7172 2025 1826 4552 2326 2838 1919 1010
SB11 , 3743 4953 1818 1925 3058 3952 2581 1038 1921 1016
SB12 , 4348 5353 6671 2525 2525 5252 1822 3838 1819 0000
SB13 , 3639 5153 0000 0000 2628 4949 1922 2838 1923 1010
SB14 , 3841 5253 7071 2025 1825 4752 1925 2238 1919 1010
SB15 , 4848 4949 1818 1925 2540 3952 2835 2828 1921 1016
SB16 , 3743 4951 1818 0000 2658 3950 2222 0000 1926 1010
SB17 , 3639 5253 7071 2525 2628 4949 1922 0000 1919 1010
SB18 , 3846 5252 7379 2525 2526 4547 2122 3838 1919 1010
SB19 , 3344 5151 7173 2025 2558 4152 2227 3838 1923 1010

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SB20 , 3951 4951 1820 2030 2454 3043 2348 1024 1921 1010
SB21 , 3849 5153 6673 2525 1830 5252 2627 1224 1919 1011
SB22 , 4244 5151 6671 2021 3030 5252 1919 1438 1923 1010
SB23 , 4243 5253 6679 2026 1825 4252 2225 2829 1823 1011
SB24 , 3638 5151 5475 2525 2626 4850 2225 1229 1819 1011
SB25 , 3843 5153 0000 2025 1826 4956 1925 1238 1923 1010
SB26 , 4346 5151 7173 2125 2530 5052 1925 3038 1823 1010
SB27 , 3842 5252 0000 2525 2530 3850 2225 1238 1819 1010
SB28 , 3846 5151 7070 2525 2426 4950 2222 3838 1823 1010
SB29 , 3839 5151 6671 2020 2628 4952 1922 2828 1923 1010
SB30 , 3840 5151 7079 2025 2425 5252 2226 2839 1923 1010
SB31 , 3844 5153 7079 2025 1826 4949 1922 3838 1923 1010
SB32 , 3839 5151 7172 2025 1824 4952 1922 2828 1923 1010
SB33 , 3842 5253 7071 2020 1818 4045 1825 2838 1919 1010
SB34 , 3243 5253 7578 2025 1658 3850 1825 1422 1923 1010
SB35 , 3738 4951 1818 1925 1840 3952 1935 1014 1926 1016
SB36 , 3640 5152 7079 2125 2630 4352 2326 3838 1819 1010
SB37 , 4242 5151 5466 2525 1824 4050 1824 2136 1819 0000
SB38 , 4242 5253 6670 1919 1818 4045 1825 2837 1919 1011
SB39 , 3846 5253 7071 2020 1826 4145 1825 1437 1919 1010
SB40 , 4344 5151 7378 2525 1858 3848 2525 3737 1923 1010
SB41 , 4042 5153 7179 2025 2528 4052 2526 1438 1519 1010
SB42 , 3846 5151 7172 2525 2426 4852 2326 2828 1919 1010
SB43 , 3644 5253 7079 2525 1824 4949 1922 2837 1923 1010
SB44 , 3643 5151 5470 2525 2530 5050 2225 2932 1819 1011
SB45 , 3838 0000 6675 2525 1818 3852 2525 1228 1919 1010
SB46 , 3846 5151 6672 2525 2426 4548 2526 1437 1919 1010
SB47 , 4346 5353 6682 2024 2526 4052 1827 1437 1919 1010
SB48 , 4242 5153 6679 2025 2528 4052 2326 1414 1519 1010
SB49 , 3643 5353 6670 2025 2325 4047 2325 3737 2323 1010
SB50 , 3636 5253 7182 2526 1825 4757 1925 3737 1923 1010
pop
BB01 , 3640 5253 5470 2525 1528 3848 2226 1414 0000 1010
BB02 , 3644 5153 7073 2025 1924 4548 2527 2837 1923 1011
BB03 , 4343 5151 7379 2020 1624 4949 1922 2839 1823 1010
BB04 , 4348 0000 7070 2026 2428 4552 1925 2438 1823 1010
BB05 , 4243 5353 7070 2526 2230 4952 1926 0000 1718 1010
BB06 , 4142 5353 7378 2026 2728 4749 2525 2238 1919 0910
BB07 , 4042 5353 5473 2526 1828 3865 2325 2238 1920 1010
BB08 , 3642 4949 1871 2030 1518 3052 2348 1039 1523 1011
BB09 , 4343 4952 5475 2024 1830 5252 1825 1438 1719 1010
BB10 , 4349 4951 5454 2020 1822 5252 1819 2539 1923 1010
BB11 , 3642 5151 8282 2025 2426 4545 1621 2938 1923 1010
BB12 , 4343 5353 6681 2026 1626 4549 1925 2832 1923 1010
BB13 , 4242 5153 7379 2525 1824 4252 2324 3838 1919 0910
BB14 , 4349 0000 5454 2026 1822 4952 1922 2425 0000 1010
BB15 , 4344 5151 7273 2020 1626 4549 2527 2838 1919 1010
BB16 , 4149 5151 6673 2526 2830 4652 2223 2838 1919 1010
BB17 , 4343 5151 7081 2020 1625 4952 2225 1212 1819 1010
BB18 , 4349 5252 7073 2526 1518 4652 2325 0000 1919 1010
BB19 , 4043 5151 7073 2025 1528 4652 2328 3438 1919 1010
BB20 , 0000 5353 0000 0000 1828 4045 2125 1439 1919 0910

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BB21 , 3648 5153 7081 2026 2828 4549 2225 2539 0000 1010
BB22 , 3642 4949 0000 2025 1524 5252 1925 2839 0000 1011
BB23 , 4243 5252 7575 2026 2528 4646 2325 3032 1919 1010
BB24 , 4343 5151 5473 2020 1616 4949 2225 3738 1923 1010
BB25 , 3648 5151 7071 2026 2529 4748 2425 2238 1919 1010
BB26 , 4848 5151 6670 2026 1828 4546 1822 2438 0000 1010
BB27 , 4041 5151 5479 2025 2429 4552 2526 2832 0000 1010
BB28 , 4343 4952 5475 2026 1830 5252 1824 1437 1519 1010
BB29 , 4048 5152 6681 2020 2930 4852 2525 3037 1823 1010
BB30 , 4346 5153 5464 2525 1822 4752 2023 1437 1717 1010
BB31 , 4041 5252 6767 2026 1818 3852 0000 3030 2020 1010
BB32 , 4349 5353 5473 2526 1618 3865 0000 0000 2020 0000
BB33 , 4249 4951 5479 2020 1824 5252 1818 2538 1717 0910
BB34 , 4143 5151 7373 2025 2830 4974 2727 0000 2424 0000
BB35 , 3641 5151 7075 2629 1930 4048 2424 0000 1821 0000
BB36 , 4346 5151 7379 2125 0000 4046 2225 2428 2020 1010
BB37 , 3641 5151 7079 2025 1518 4952 2225 2430 1919 1010
BB38 , 3640 0000 7982 2020 2526 4652 1925 2930 1823 1010
BB39 , 4448 5151 6670 2025 2629 3852 2223 1437 1919 1010
BB40 , 3642 5253 5466 2026 1525 4646 2125 2730 1923 1010
BB41 , 4242 5151 7070 2026 1825 3849 2525 3738 1719 1010
BB42 , 0000 5151 0000 0000 2529 5265 2526 1432 1919 1010
BB43 , 3646 5153 7070 2025 2528 4252 2225 3737 1719 1010
BB44 , 4048 5151 6681 2525 2629 4652 2525 3037 1919 0910
BB45 , 4044 5253 6679 2021 2526 4546 2528 2237 1919 1010
BB46 , 3646 5253 6671 2025 2026 4648 1925 3037 1819 1010
BB47 , 3851 5153 5487 2526 2529 5265 2525 1432 1919 1010
BB48 , 3740 5151 7079 2025 1525 4346 2125 1430 2223 1010
BB49 , 3643 5151 7575 2025 1928 3848 2123 1437 1919 0910
BB50 , 4042 5151 7070 2025 1818 3840 2325 3737 1919 1010
BB51 , 4343 5152 0000 2025 1525 4652 0000 0000 0000 0000
BB52 , 3639 5353 5454 2526 2528 4652 0000 0000 0000 0000
BB53 , 4043 5151 5466 2525 2425 3852 0000 0000 0000 0000
BB54 , 4648 5153 7073 2026 1818 4949 0000 0000 0000 0000
BB55 , 3742 4953 5571 2530 1839 3049 0000 0000 0000 0000
BB56 , 3643 5152 6670 2525 1830 5252 0000 0000 0000 0000
BB57 , 3643 5253 0000 2025 1618 0000 0000 0000 0000 0000
BB58 , 4243 5353 5454 2526 2428 4652 0000 0000 0000 0000
BB59 , 3641 5353 0000 2525 1824 4864 0000 0000 0000 0000
BB60 , 4344 4951 0000 2020 1826 3848 0000 0000 0000 0000
BB61 , 4344 5151 0000 2026 1824 3849 0000 0000 0000 0000
BB62 , 3848 5153 7073 2526 1818 4152 0000 0000 0000 0000
BB63 , 3640 5152 0000 2525 1826 5252 0000 0000 0000 0000
BB64 , 4043 5151 0000 2531 1525 0000 0000 0000 0000 0000
BB65 , 4042 5151 0000 2525 1618 5252 0000 0000 0000 0000
BB66 , 4343 5152 0000 2020 0000 4646 0000 0000 0000 0000
BB67 , 3638 5151 6770 2020 1818 4852 0000 0000 0000 0000
BB68 , 3640 5151 7079 2525 1628 5252 0000 0000 0000 0000
BB69 , 4346 5151 7179 2020 1830 3852 0000 0000 0000 0000
BB70 , 4243 5153 7279 2025 0000 4152 0000 0000 0000 0000
BB71 , 4343 5151 6676 2026 1824 3852 0000 0000 0000 0000
BB72 , 3636 5153 7373 2525 2424 4357 0000 0000 0000 0000

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BB73 , 4243 5153 5473 2525 1818 5052 0000 0000 0000 0000
BB74 , 4041 5151 0000 0000 0000 3852 0000 0000 0000 0000
BB75 , 3638 5252 0000 0000 1826 4152 0000 0000 0000 0000
BB76 , 3646 4953 0000 2020 1930 4852 0000 0000 0000 0000
BB77 , 4244 5153 6673 2526 2425 5265 0000 0000 0000 0000
BB78 , 3842 5353 5470 2526 1827 4652 0000 0000 0000 0000
BB79 , 4043 5153 5466 2020 1619 4045 0000 0000 0000 0000
BB80 , 0000 5151 7070 2526 1818 5252 0000 0000 0000 0000
BB81 , 3643 5151 5475 2025 0000 3852 0000 0000 0000 0000
BB82 , 3236 5152 7072 2025 2124 4252 0000 0000 0000 0000
BB83 , 0000 5151 5454 2020 1829 3852 0000 0000 0000 0000
BB84 , 4649 5353 0000 2126 1628 4852 0000 0000 0000 0000
BB85 , 3842 5153 0000 0000 2525 4352 0000 0000 0000 0000
BB86 , 3848 5153 7373 2526 1818 4652 0000 0000 0000 0000
BB87 , 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000
BB88 , 3640 5151 7070 2025 1618 3852 0000 0000 0000 0000
BB89 , 0000 0000 6781 2526 2528 3846 0000 0000 0000 0000
BB90 , 3848 5353 0000 2020 1818 4152 0000 0000 0000 0000
BB91 , 4346 5153 0000 2125 1828 4652 0000 0000 0000 0000
BB92 , 4243 5152 0000 2525 2020 3843 0000 0000 0000 0000
BB93 , 3636 5152 0000 2525 1828 5252 0000 0000 0000 0000
BB94 , 4649 5353 0000 2026 1625 4665 0000 0000 0000 0000
BB95 , 4446 5152 7177 2020 1625 5252 0000 0000 0000 0000
BB96 , 0000 4949 8182 2526 1825 4650 0000 0000 0000 0000
BB97 , 3643 5151 0000 2025 1818 5252 0000 0000 0000 0000
BB98 , 3942 5151 0000 2525 2525 0000 0000 0000 0000 0000
BB99 , 4344 5151 0000 2025 1818 4852 0000 0000 0000 0000
BB100 , 4449 0000 0000 2126 0000 0000 0000 0000 0000 0000
pop
LA02 , 4243 5151 7070 2025 2626 4848 2325 2838 1818 1010
LA03 , 3646 5353 0000 2025 3040 4952 2223 2537 1919 1010
LA04 , 3242 5151 6666 2525 1825 5252 0000 0000 0000 0000
LA05 , 3843 5151 7087 2526 1838 4045 2225 3737 1919 1010
LA06 , 4246 5252 6472 2025 1830 5052 2527 2832 1923 1010
LA07 , 4046 5151 7173 2526 1830 5252 2325 1238 1919 1010
LA08 , 3648 5151 6670 2025 1518 5073 1621 2937 1923 1010
LA09 , 3239 5152 5466 2025 1825 4352 2527 0000 1919 0910
LA10 , 3243 5253 7273 2526 1818 3843 2527 2237 1819 0910
LA11 , 4246 5252 5472 2025 2430 5052 2527 3237 1923 1010
LA12 , 3646 5151 7279 2025 1818 3848 2526 3737 1519 1010
LA13 , 3846 5152 5464 2025 2528 3852 2526 3738 1919 1010
LA14 , 3636 5152 5473 2026 1626 4849 2121 3032 1919 1010
LA15 , 3746 5153 7175 2525 2525 3857 2327 3737 1919 1010
LA16 , 3643 5151 7073 2020 2530 4052 2325 0000 1919 1010
LA17 , 3244 5151 7879 2026 2530 4952 1821 3233 1919 1010
LA18 , 3642 5151 7073 2025 1838 5273 2123 1230 1919 1010
LA19 , 4046 5153 7175 2525 2530 3852 0000 3737 1919 1010
LA20 , 4046 5252 7071 2020 1825 5252 2525 3032 1919 1010
LA21 , 4346 5153 7079 2025 1838 4052 2325 3038 1919 1010
LA22 , 3242 5151 7171 2026 2525 3840 2323 1228 1919 1010
LA23 , 4244 5152 5466 0000 1825 4352 2627 3737 1923 1010
LA24 , 4246 5153 5471 2525 1830 4650 1627 2830 1919 1010

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LA25 , 4346 5151 5473 2626 2528 3840 2525 1237 1919 1010
LA26 , 3843 5152 7073 2026 1628 4057 2325 1425 1919 1010
LA27 , 3636 5151 5454 2525 2538 4873 2123 2230 1919 1010
LA28 , 4142 5151 7879 2026 2525 4048 2125 3238 1919 1010
LA29 , 3643 5151 7073 2525 1838 4872 2123 1230 1919 1010
LA30 , 3242 5151 7173 2026 2425 4049 2526 1214 1919 1010
LA31 , 4343 5152 7078 2025 2530 5252 1925 3030 1919 1010
LA32 , 4646 5151 6671 2025 3030 4652 2527 3037 1919 1010
LA33 , 4346 5152 7071 2525 1818 4952 1926 3738 1919 1010
LA34 , 4246 5152 5466 2026 2525 5252 1925 3237 1923 0910
LA35 , 3243 5151 7073 2025 2538 4242 2123 1237 1919 1010
LA36 , 4243 5153 7172 2126 1826 4852 2223 3838 1919 1010
LA37 , 3642 5151 5467 2526 2538 3845 2325 3839 1819 1010
LA38 , 4042 5151 7079 2525 3038 3838 2225 0000 1919 1010
LA39 , 4646 5153 7175 2525 1830 3857 2323 1238 1919 1010
LA40 , 3641 5151 5473 2020 2630 4952 2526 3737 1919 0910
LA41 , 3643 5153 7073 2025 1825 3840 2526 3737 1919 0910
LA42 , 3842 5253 7072 2026 1818 4048 2225 3737 1919 1010
LA43 , 0000 5153 5454 2525 0000 0000 2626 1414 1515 1010
LA44 , 3242 5153 7287 2020 2630 4052 1927 1237 1923 1010
LA45 , 4044 5353 5471 2526 1830 3857 2325 3737 1923 1010
LA46 , 3843 5152 6673 2020 2428 4052 2125 2525 1919 1010
LA47 , 4444 5151 5466 2626 1818 4852 2325 1232 1919 1010
LA48 , 4646 5153 6673 2525 1830 4052 1719 3738 1819 1010
LA49 , 3843 5151 7071 2525 1818 5252 2126 2837 1819 1010
LA50 , 3842 5151 7879 2026 1828 4048 2226 3737 1923 1011
LA51 , 0000 0000 6673 2525 0000 2828 2225 3738 1919 1010
LA52 , 3242 5153 5454 2026 2525 4049 1821 3238 1919 1010
LA53 , 3741 5153 6470 2126 3038 5273 0000 0000 0000 0000
LA54 , 3644 5153 6672 2025 1825 3840 2526 3737 1919 1010
LA55 , 3642 5151 7070 2126 3838 4952 1819 1438 1823 1010
pop
YC01 , 3536 4949 7282 2121 2424 3434 3336 0000 1919 0000
YC02 , 3646 4949 7171 2121 2224 3333 3336 0000 1919 0000
YC03 , 3546 4949 8082 2121 2224 3333 3636 0000 1919 0000
YC04 , 3646 4949 7180 2121 2224 3333 3638 0000 1819 0000
YC05 , 3646 4949 7780 2121 2424 3334 3638 0000 1818 0000
YC06 , 3636 4949 8082 2121 2424 3434 3336 0000 1919 0000
YC07 , 3636 4949 8082 2121 2224 3333 3336 0000 1919 0000
YC08 , 3636 4949 7180 2121 2224 3334 3535 0000 1818 0000
YC09 , 3636 4949 8082 2121 2424 3334 0000 0000 1819 0000
YC10 , 4646 4949 7182 2121 2222 3434 3638 0000 1919 0000
YC11 , 3536 4949 8082 2121 2222 3434 0000 0000 0000 0000
YC12 , 3636 4949 8080 2121 2424 3333 3838 0000 1819 0000
YC13 , 3646 4949 8082 2121 2424 3334 3636 0000 1919 0000
YC14 , 3546 4949 7180 2121 2224 3435 3636 0000 1819 0000
YC15 , 3636 4949 7180 2121 2424 3434 3636 0000 1919 0000
YC16 , 3636 4949 8080 2121 2222 3434 3537 0000 1819 0000
YC17 , 3636 4949 7182 2121 2224 3333 0000 0000 1919 0000
YC18 , 3636 4949 7171 2121 2224 3434 3535 0000 1919 0000
YC19 , 3535 4949 7180 2121 2222 3333 3535 0000 1819 0000
YC20 , 3436 4949 7171 2121 2224 3434 3636 0000 1819 0000

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YC21 , 3546 4949 8080 2121 2224 3333 3636 0000 1819 0000
YC22 , 3536 4949 8080 2121 2224 3333 3535 0000 1818 0000
YC23 , 3646 4949 7780 2121 2222 3333 3636 0000 1819 0000
YC24 , 3646 4949 8080 2121 2224 3434 3636 0000 1819 0000
YC25 , 3535 0000 0000 2121 0000 3333 0000 0000 1919 0000

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APPENDIX D. ALLELE FREQUENCIES FOR 13 WASHINGTON COASTAL CUTTHROAT TROUT POPULATIONS AT 6 MICROSATELLITE LOCI. NUMBERS OF ALLELES SCORED (2*N*) ARE GIVEN FOR EACH POPULATION/LOCUS COMBINATION. FRAGMENT SIZES ARE GIVEN IN BASES (B).

Locus	(b) Allele	Population												
		Parker	Covington	Fennel	Double Ditch	Gold	Stavis	Gierin	Peabody	Goodman	Snahapish	Oxbow	Wildcat	Salt
Omy77	107 30	0.011	0	0	0	0	0	0	0	0	0	0	0	0
	109 31	0	0	0	0.011	0	0	0.009	0	0.052	0.088	0.042	0	0.012
	111 32	0.096	0.100	0.063	0.074	0	0.060	0	0.094	0.224	0.147	0.104	0	0.159
	113 33	0.043	0.043	0	0	0	0.012	0	0	0	0	0.042	0	0.085
	115 34	0	0	0	0	0	0	0	0	0	0	0	0	0
	117 35	0	0	0	0	0	0.012	0	0	0	0	0	0	0
	119 36	0.383	0.114	0.125	0.011	0.222	0.202	0.064	0.198	0	0.029	0	0	0.098
	121 37	0	0.057	0.013	0.021	0.009	0.095	0	0	0.017	0.206	0	0.023	0.122
	123 38	0.011	0.043	0.162	0	0.074	0.024	0.027	0	0.086	0	0	0	0.061
	125 39	0	0	0.050	0.011	0.009	0.024	0	0	0	0	0.083	0	0
	127 40	0	0.129	0.013	0	0.065	0.095	0.027	0	0	0	0.021	0	0.037
	129 41	0.011	0.200	0.188	0.181	0.056	0.107	0.245	0.047	0	0	0.104	0	0.183
	131 42	0.011	0	0.013	0.106	0.074	0.012	0.236	0	0	0.108	0.271	0.091	0.012
	133 43	0.128	0.043	0.237	0.330	0.287	0.262	0.082	0	0.207	0.069	0	0.250	0
	135 44	0.245	0.129	0.100	0.181	0.083	0.012	0.082	0.509	0.017	0	0.125	0.068	0.098
	137 46	0	0	0.013	0	0.083	0.024	0.155	0	0.190	0.255	0.083	0.091	0
	139 48	0	0.029	0	0.011	0	0	0.009	0.028	0.138	0	0	0	0.073
	141 49	0.053	0.029	0	0.064	0.028	0.012	0.064	0.123	0.017	0.098	0	0.432	0.012
	143 50	0.011	0.071	0.025	0	0	0	0	0	0	0	0	0	0.049
	145 51	0	0	0	0	0.009	0.048	0	0	0	0	0.042	0	0
	147 52	0	0	0	0	0	0	0	0	0	0	0.021	0	0
	153 55	0	0	0	0	0	0	0	0	0	0	0	0.023	0
	155 56	0	0.014	0	0	0	0	0	0	0.052	0	0	0.023	0
	159 58	0	0	0	0	0	0	0	0	0	0	0.063	0	0

Locus	Allele													
2N		94	70	80	94	108	84	110	106	58	102	48	44	82
Oneu11	144 49	0	0	0	0.010	0	0.037	0.070	0.018	0.065	0.200	0	0	0
	146 51	0.128	0.574	1.000	0.615	0.798	0.573	0.395	0.759	0.694	0.436	0.813	1.000	0.632
	147 52	0.745	0.250	0	0.094	0.096	0.110	0	0	0.065	0.345	0.083	0	0.184
	148 53	0.128	0.176	0	0.281	0.106	0.280	0.535	0.223	0.177	0.018	0.104	0	0.184
	149 54	0	0	0	0	0	0	0	0	0	0	0	0	0
2N		94	68	84	96	104	82	114	112	62	110	48	48	76
Ots1	231 50	0	0	0.068	0	0	0	0	0	0.103	0	0	0	0
	235 52	0	0	0	0	0	0	0	0	0	0.031	0	0	0
	237 53	0	0.015	0	0	0	0	0	0	0	0	0	0	0
	239 54	0	0.059	0.341	0	0	0.013	0.018	0	0	0	0	0	0.058
	241 55	0	0	0	0	0	0	0	0	0	0	0	0	0
	243 56	0.125	0	0	0	0	0	0	0	0	0	0.087	0.114	0
	245 57	0	0	0	0	0	0	0.061	0	0	0	0	0	0
	249 59	0	0	0	0	0	0	0.018	0	0	0	0	0	0
	253 61	0	0.044	0	0	0	0	0	0	0.017	0	0	0	0.019
	255 62	0	0	0	0	0	0	0	0	0.086	0.224	0	0.045	0
	259 64	0.031	0	0	0	0.019	0	0.079	0	0	0	0.022	0.045	0
	261 65	0	0.044	0	0	0	0	0	0.010	0	0	0.022	0	0.154
	263 66	0.094	0.074	0.250	0.381	0.163	0.013	0	0.040	0	0	0.283	0	0.077
	265 67	0.031	0	0	0.214	0.067	0.051	0.175	0	0	0	0.130	0.023	0.038
	267 68	0.063	0.015	0	0	0	0.013	0	0.040	0.034	0	0.065	0	0
	269 69	0	0	0.023	0	0	0	0.035	0.020	0.086	0	0	0.227	0
	271 70	0.156	0.059	0.114	0.048	0.173	0.282	0.079	0.440	0	0	0	0	0.019
	273 71	0	0.147	0	0	0.106	0.103	0.096	0.260	0	0.010	0.022	0.068	0.115
	275 72	0	0	0	0.048	0.192	0.038	0.061	0	0.103	0.051	0	0	0
	277 73	0	0.412	0	0.167	0.058	0.154	0.175	0.190	0.034	0.020	0.022	0.091	0.442
	279 74	0	0.015	0	0.024	0	0	0	0	0.017	0	0.022	0	0
	281 75	0.031	0	0	0	0.029	0.090	0.009	0	0.052	0.214	0.261	0.227	0
	283 76	0.375	0	0.023	0	0	0.013	0	0	0.121	0.031	0	0.114	0
	285 77	0	0.029	0	0	0	0	0	0	0	0.133	0.022	0	0
	287 78	0.031	0.029	0	0	0	0	0.070	0	0	0	0	0	0.019

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	289 79	0	0.029	0.136	0	0.058	0.141	0	0	0	0	0	0.045	0.019
	291 80	0	0.015	0	0	0	0.013	0	0	0	0	0	0	0
	293 81	0.031	0	0	0.048	0.096	0.038	0.123	0	0	0	0.022	0	0.019
	295 82	0.031	0	0	0.071	0.010	0.038	0	0	0	0.122	0	0	0
	297 83	0	0	0	0	0.019	0	0	0	0.086	0.153	0.022	0	0
	299 84	0	0	0.045	0	0	0	0	0	0	0	0	0	0
	301 85	0	0.015	0	0	0	0	0	0	0.259	0	0	0	0.019
	305 87	0	0	0	0	0.010	0	0	0	0	0	0	0	0
	311 90	0	0	0	0	0	0	0	0	0	0.010	0	0	0
2N		32	68	44	42	104	78	114	100	58	98	46	44	52
Ots4	101 15	0	0	0	0	0.009	0	0.209	0	0	0	0	0	0
	103 16	0	0	0	0	0.009	0	0	0	0	0	0	0	0
	109 19	0	0	0	0.010	0.057	0	0	0	0	0	0	0	0.015
	111 20	0.053	0.181	0.048	0.385	0.160	0.256	0.093	0.750	0.417	0.422	0.563	0.273	0.279
	113 21	0	0.014	0	0	0.019	0.012	0.128	0.102	0.017	0.029	0	0.136	0.088
	115 22	0	0	0	0	0.132	0	0.047	0	0	0	0	0	0
	117 23	0	0	0	0	0.009	0	0.081	0	0.033	0	0.104	0	0.015
	119 24	0.011	0	0.024	0	0.009	0	0.047	0	0	0	0	0	0
	121 25	0.606	0.667	0.690	0.479	0.519	0.500	0.221	0.148	0.183	0.314	0.125	0.364	0.515
	123 26	0.330	0.083	0.226	0.115	0.075	0.085	0.174	0	0.300	0.196	0.042	0.068	0.029
	125 27	0	0.014	0.012	0	0	0.049	0	0	0	0.039	0	0	0
	127 28	0	0.014	0	0.010	0	0.049	0	0	0.050	0	0.167	0.159	0.059
	129 29	0	0.014	0	0	0	0.049	0	0	0	0	0	0	0
	131 30	0	0.014	0	0	0	0	0	0	0	0	0	0	0
	133 31	0	0	0	0	0	0	0	0	0	0	0	0	0
2N		94	72	84	96	106	82	86	108	60	102	48	44	68
Sfo8	192 15	0	0	0.053	0	0.010	0.095	0.096	0	0.083	0	0.021	0.217	0.012
	194 16	0.135	0.014	0.053	0.067	0.010	0.024	0	0	0.083	0	0	0	0
	196 17	0	0	0	0.017	0	0	0	0	0	0	0	0	0
	198 18	0.058	0.114	0.237	0.083	0.221	0.238	0.061	0.019	0	0	0.104	0	0.105
	200 19	0.096	0.057	0	0.033	0.058	0.012	0.132	0.288	0.117	0.010	0	0.022	0.058
	202 20	0	0	0.158	0	0	0	0	0	0	0	0.042	0.043	0.023

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204 21	0	0	0	0	0	0	0	0.010	0	0	0	0	0
206 22	0	0	0	0	0.010	0	0	0	0.050	0.133	0	0.043	0
208 23	0.019	0	0	0.017	0	0.012	0	0	0	0	0	0.130	0
210 24	0.212	0.229	0.158	0.167	0.183	0.179	0.079	0.115	0.050	0.082	0.271	0.239	0.221
212 25	0.038	0.057	0.026	0.200	0.221	0.190	0	0.221	0.050	0.163	0.063	0.087	0.058
214 26	0.308	0.100	0.289	0.017	0.058	0.024	0.281	0.144	0.300	0.316	0.354	0.109	0.116
216 27	0	0.043	0.026	0.033	0	0.012	0.035	0.183	0.083	0	0	0	0.023
218 28	0.038	0.057	0	0.100	0.115	0.095	0.140	0	0.033	0.010	0.083	0.065	0
220 29	0.077	0.043	0	0.117	0	0	0.009	0	0	0	0	0	0.140
222 30	0	0.157	0	0.117	0.019	0.107	0.140	0.019	0	0	0.021	0	0.174
224 31	0	0.114	0	0.017	0	0	0	0	0	0	0	0	0.058
226 32	0	0.014	0	0	0	0	0	0	0	0	0	0	0
228 33	0	0	0	0	0	0	0.026	0	0	0	0.021	0	0
230 34	0	0	0	0	0	0	0	0	0	0	0	0	0
234 36	0	0	0	0	0	0	0	0	0	0.102	0	0	0
238 38	0	0	0	0	0.048	0.012	0	0	0.033	0.143	0	0.043	0
240 39	0	0	0	0.017	0.048	0	0	0	0.033	0	0	0	0
242 40	0.019	0	0	0	0	0	0	0	0.050	0	0	0	0.012
244 41	0	0	0	0	0	0	0	0	0.033	0.041	0.021	0	0
2N	52	70	38	60	104	84	114	104	60	98	48	46	86
Ssa85 103 27	0	0	0	0	0	0	0	0	0	0	0	0	0.014
105 28	0	0	0	0	0	0	0	0	0	0	0	0	0
109 30	0	0	0	0.011	0	0	0	0	0	0	0	0	0
111 31	0	0	0	0	0.020	0	0	0	0	0	0	0	0
115 33	0	0.061	0	0.021	0	0.024	0	0	0.016	0	0.020	0	0.054
117 34	0	0	0	0	0	0	0	0	0.125	0	0	0	0
119 35	0	0	0	0	0	0	0	0	0	0.179	0.080	0.045	0
123 37	0	0	0	0.043	0	0	0	0	0	0	0	0	0
125 38	0	0.061	0.073	0	0.010	0.012	0.080	0	0.047	0	0.120	0	0.041
127 39	0	0	0	0.213	0	0	0	0	0	0	0	0	0
129 40	0.021	0.015	0.049	0.043	0.069	0.119	0	0	0	0	0	0	0.027
131 41	0	0.015	0.159	0	0.010	0.024	0	0	0	0.019	0.020	0	0
133 42	0.011	0	0	0	0	0.036	0.054	0	0.031	0.104	0.020	0	0

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135 43	0.085	0.152	0.110	0	0.088	0.179	0.134	0.588	0	0	0	0	0.095
137 44	0	0	0	0.128	0	0	0	0	0.063	0.038	0	0.273	0
139 45	0	0.197	0.171	0	0.039	0.143	0.063	0.020	0	0	0.120	0.023	0.257
141 46	0.096	0.121	0	0.266	0.255	0.143	0	0	0	0.038	0.020	0.068	0.203
143 47	0.511	0.091	0	0	0	0.024	0	0.039	0	0	0.160	0.227	0.068
145 48	0.266	0.106	0.061	0.011	0.049	0.036	0.232	0	0	0	0.120	0.023	0.108
147 49	0	0	0.085	0.011	0.029	0.012	0.063	0.314	0.188	0.226	0	0	0
149 50	0.011	0	0	0	0.010	0	0	0	0	0	0	0	0
151 51	0	0.015	0	0	0	0	0.080	0.039	0.016	0	0	0	0.027
153 52	0	0.015	0.195	0.032	0.225	0.131	0	0	0.063	0	0	0	0.014
155 53	0	0	0.061	0.021	0	0	0	0	0	0	0	0	0
157 54	0	0.076	0	0.032	0	0.012	0	0	0	0	0	0.068	0.041
159 55	0	0.030	0	0	0	0	0	0	0.016	0	0	0	0
161 56	0	0	0	0.043	0.059	0.012	0.295	0	0.031	0	0	0	0
163 57	0	0	0	0.117	0	0.012	0	0	0	0	0	0.023	0
167 59	0	0	0	0	0	0	0	0	0.047	0.009	0	0	0
169 60	0	0	0.037	0	0	0.012	0	0	0	0	0.080	0.045	0
171 61	0	0	0	0	0.088	0	0	0	0.031	0.075	0.040	0	0
173 62	0	0	0	0	0	0	0	0	0.172	0.047	0.020	0.114	0
175 63	0	0	0	0.011	0	0	0	0	0	0	0.080	0.068	0
177 64	0	0	0	0	0.010	0	0	0	0	0	0	0	0
179 65	0	0.045	0	0	0.020	0.012	0	0	0	0	0	0	0.054
183 67	0	0	0	0	0.010	0	0	0	0	0	0	0	0
187 69	0	0	0	0	0	0	0	0	0	0	0.040	0	0
189 70	0	0	0	0	0	0	0	0	0	0.009	0.020	0	0
191 71	0	0	0	0	0	0	0	0	0.063	0.038	0	0	0
193 72	0	0	0	0	0	0	0	0	0	0.094	0	0	0
195 73	0	0	0	0	0	0	0	0	0.047	0.028	0	0	0
197 74	0	0	0	0	0.010	0.060	0	0	0	0.009	0.040	0	0
199 75	0	0	0	0	0	0	0	0	0.031	0	0	0	0
201 76	0	0	0	0	0	0	0	0	0.016	0	0	0	0
203 77	0	0	0	0	0	0	0	0	0	0.075	0	0.023	0
205 78	0	0	0	0	0	0	0	0	0	0.009	0	0	0
2N	94	66	82	94	102	84	112	102	64	106	50	44	74

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APPENDIX E. ALLELE FREQUENCIES FOR 10 HOOD CANAL COASTAL CUTTHROAT TROUT POPULATIONS AT 10 MICROSATELLITE LOCI (OTS101 EXCLUDED DUE TO A HIGH PERCENTAGE OF APPARENT NULL ALLELES) AND ONE YELLOWSTONE POPULATION AT 8 LOCI. NUMBERS OF ALLELES SCORED (2*N*) ARE GIVEN FOR EACH POPULATION/LOCUS COMBINATION. FRAGMENT SIZES ARE IN BASES (B). LOCUS/POPULATION COMBINATIONS FOR WHICH AMPLIFICATION WAS NOT ATTEMPTED ARE MARKED WITH "-". PRIVATE ALLELE FREQUENCIES ARE IN BOLD.

Locus	(b)	Population										
		Thorndyke	Tarboo	Fulton	Courtney	Bear	Big Mission	Stavis	Seabeck	Big Beef	Little Anderson	Yellowstone
Omy77	111	0.011	0.015	0	0	0	0	0	0	0.005	0.087	0
	113	0.16	0.03	0.289	0.043	0.037	0.025	0.022	0.013	0	0	0
	115	0	0	0	0	0	0	0	0	0	0	**0.020**
	117	0	0.015	0	0.152	0.019	0.162	0	0	0	0	0.200
	119	0.234	0.015	0	0.087	0	0.2	0.144	0.128	0.163	0.144	0.560
	121	0	0	0	0	0	0.038	0.044	0	0.011	0.019	0
	123	0.106	0.076	0	0.022	0.056	0.025	0.044	0.256	0.043	0.067	0
	125	0	0	0	0.022	0.019	0.05	0.011	0.051	0.011	0.01	0
	127	0.053	0.03	0.105	0.13	0.167	0.075	0.189	0.077	0.103	0.048	0
	129	0.064	0.015	0	0.022	0.019	0	0.089	0.013	0.049	0.029	0
	131	0.043	0.152	0.026	0.152	0.204	0.05	0.122	0.154	0.125	0.183	0
	133	0.181	0.136	0.211	0.217	0.167	0.237	0.111	0.103	0.255	0.154	0
	135	0.074	0.167	0.263	0.13	0.148	0.1	0.033	0.064	0.054	0.058	0
	137	0.032	0.076	0	0.022	0.037	0	0.067	0.103	0.06	0.192	0.220
	139	0	0.015	0	0	0	0	0	0.026	0.065	0.01	0
	141	0	0.227	0	0	0.074	0.025	0.033	0.013	0.049	0	0
	143	0.011	0	0	0	0	0	0	0	0	0	0

	145	0.032	0.015	0	0	0.056	0.013	0.089	0	0.005	0	0
	147	0	0.015	0	0	0	0	0	0	0	0	0
	159	0	0	0.105	0	0	0	0	0	0	0	0
2N		94	66	38	46	54	80	90	78	184	104	50
Oneu11	144	0	0	0	0	0	0	0.053	0	0.059	0	1.000
	146	0.391	0.676	0.765	0.773	0.655	0.658	0.628	0.487	0.548	0.66	0
	147	0.043	0.118	0	0.023	0.138	0.158	0.096	0.184	0.129	0.16	0
	148	0.543	0.206	0.235	0.205	0.207	0.184	0.223	0.329	0.263	0.179	0
	149	0.022	0	0	0	0	0	0	0	0	0	0
2N		92	68	34	44	58	76	94	76	186	106	48
Ots1	239	0.14	0.227	0	0.068	0.052	0.029	0.021	0.069	0.181	0.17	0
	241	0	0	0	0	0	0	0	0	0.007	0	0
	243	0	0	0.038	0	0	0	0	0	0	0	0
	255	0	0	0	0	0.052	0	0	0	0	0	0
	259	0	0	0	0	0	0	0	0	0.007	0.028	0
	261	0	0	0	0	0	0.029	0	0	0	0	0
	263	0.035	0.258	0	0.159	0.172	0.143	0.021	0.222	0.097	0.113	0
	265	0.047	0.015	0	0.159	0.086	0.086	0.117	0	0.028	0.009	0
	267	0	0	0	0	0.034	0	0	0	0	0	0
	269	0	0	0.038	0.023	0.052	0	0	0	0	0	0
	271	0.337	0.061	0	0.068	0.207	0.271	0.223	0.181	0.229	0.189	0
	273	0.047	0.182	0.115	0.091	0.138	0.186	0.138	0.222	0.035	0.132	0.271
	275	0	0.03	0.115	0.045	0.017	0.029	0.053	0.069	0.021	0.075	0.021
	277	0.302	0.106	0.077	0.068	0.069	0.043	0.16	0.056	0.153	0.142	0
	279	0	0	0	0	0.017	0.014	0	0	0	0	0
	281	0	0	0	0.023	0	0.1	0.032	0.028	0.056	0.028	0
	283	0	0	0.077	0	0	0	0.011	0	0.007	0	0
	285	0	0	0.038	0	0	0	0	0	0.007	0	0.042
	287	0	0	0	0.091	0.034	0	0.064	0.014	0.007	0.038	0
	289	0.07	0.03	0.038	0.045	0.034	0.014	0.064	0.111	0.083	0.057	0
	291	0	0.015	0	0	0	0	0	0	0	0	0.479
	293	0	0.076	0.077	0.023	0	0.057	0.043	0	0.049	0	0

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	295	0.012	0	0.269	0	0	0	0.053	0.028	0.028	0	0.188
	297	0	0	0.115	0	0.017	0	0	0	0	0	0
	305	0.012	0	0	0.136	0.017	0	0	0	0.007	0.019	0
2N		86	66	26	44	58	70	94	72	144	106	48
Ots4	109	0	0	0.029	0	0	0.013	0	0.027	0	0	0
	111	0.404	0.471	0.265	0.333	0.611	0.184	0.25	0.284	0.392	0.33	0
	113	0.043	0	0.118	0.063	0.019	0.053	0.033	0.041	0.027	0.028	1.000
	115	0.011	0	0	0	0	0.053	0	0	0	0	0
	119	0	0	0	0	0.056	0.026	0	0.014	0.005	0	0
	121	0.436	0.357	0.588	0.583	0.222	0.474	0.489	0.608	0.376	0.434	0
	123	0.096	0.143	0	0.021	0.093	0.145	0.228	0.027	0.183	0.208	0
	125	0.011	0	0	0	0	0.053	0	0	0	0	0
	127	0	0.029	0	0	0	0	0	0	0	0	0
	129	0	0	0	0	0	0	0	0	0.005	0	0
	131	0	0	0	0	0	0	0	0	0.005	0	0
	133	0	0	0	0	0	0	0	0	0.005	0	0
2N		94	70	34	48	54	76	92	74	186	106	50
Sfo8	192	0.011	0.043	0.265	0.043	0.052	0	0.043	0	0.049	0.01	0
	194	0.064	0.014	0	0.043	0.052	0.014	0.064	0.013	0.082	0.019	0
	196	0	0	0	0.087	0.017	0	0	0	0	0	0
	198	0.255	0.257	0.029	0.065	0.121	0.297	0.287	0.231	0.288	0.298	0
	200	0.011	0	0.059	0	0	0.014	0.032	0	0.027	0	0
	202	0	0	0	0	0	0	0	0	0.016	0	0
	204	0	0.014	0.029	0	0	0	0	0	0.005	0	0
	206	0	0	0.059	0	0.017	0.027	0	0	0.022	0	0.458
	208	0.011	0.014	0	0	0	0	0	0.013	0	0	0
	210	0.085	0.143	0	0.13	0.19	0.041	0.117	0.09	0.092	0.029	0.542
	212	0.106	0.243	0	0.37	0.379	0.27	0.234	0.256	0.13	0.25	0
	214	0.085	0.086	0.088	0.087	0.052	0.095	0	0.205	0.06	0.058	0
	216	0	0	0.059	0	0.017	0	0.053	0	0.011	0	0
	218	0.106	0.086	0.118	0.065	0.069	0.081	0.021	0.077	0.114	0.048	0
	220	0	0	0.059	0.022	0	0	0.011	0	0.043	0	0

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	222	0.223	0.071	0.059	0	0.017	0	0.117	0.103	0.054	0.173	0
	224	0	0	0.118	0	0	0	0	0	0	0	0
	226	0	0	0.059	0	0	0	0	0	0	0	0
	228	0	0	0	0	0.017	0.054	0.011	0	0	0	0
	230	0	0	0	0	0	0.014	0	0	0	0	0
	238	0	0.029	0	0	0	0.095	0.011	0.013	0	0.106	0
	240	0.043	0	0	0.087	0	0	0	0	0.005	0	0
	242	0	0	0	0	0	0	0	0	0	0.01	0
2N		94	70	34	46	58	74	94	78	184	104	48
Ssa85	105	0	0	0	0	0	0	0	0	0	0.019	0
	109	0	0	0	0	0	0	0	0	0.005	0	0
	111	0	0	0	0	0.069	0	0	0	0	0	0
	115	0	0	0	0	0	0.013	0	0	0	0	0.520
	117	0	0	0	0	0	0	0	0	0	0	0.460
	119	0	0	0	0	0	0	0	0	0	0	0.020
	125	0	0.014	0	0.043	0.069	0.038	0.057	0.026	0.101	0.132	0
	129	0	0.043	0.029	0.13	0.069	0.075	0.08	0.115	0.027	0.151	0
	131	0	0.086	0.147	0.022	0	0.013	0.023	0.038	0.021	0	0
	133	0.033	0.014	0.118	0	0.017	0.013	0.034	0.013	0.016	0.019	0
	135	0.076	0.114	0	0.152	0.069	0.112	0.045	0.013	0.021	0.028	0
	137	0	0	0.147	0	0	0	0	0	0	0	0
	139	0.163	0.029	0	0.043	0.017	0.162	0.125	0.077	0.064	0.019	0
	141	0.272	0.014	0.029	0.065	0.034	0.038	0.193	0	0.133	0.019	0
	143	0.011	0	0.059	0	0.034	0.025	0.023	0.064	0.016	0	0
	145	0.13	0.186	0.029	0	0.069	0.1	0.102	0.077	0.069	0.104	0
	147	0.043	0.029	0	0.065	0.034	0.05	0.045	0.154	0.096	0.075	0
	149	0.033	0.029	0.118	0.022	0.017	0	0.08	0.128	0.011	0.038	0
	151	0	0	0	0.022	0	0.013	0	0	0	0	0
	153	0.12	0.343	0	0.196	0.293	0.237	0.102	0.269	0.372	0.311	0
	155	0	0.014	0.029	0	0	0	0	0	0	0	0
	159	0	0	0	0	0	0	0.011	0	0	0	0
	161	0	0	0	0.043	0.086	0.063	0.011	0.013	0	0	0
	163	0.011	0.043	0	0	0	0	0.045	0.013	0.005	0.038	0

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	167	0	0	0.206	0	0	0	0	0	0	0	0
	171	0	0	0.029	0	0.034	0	0	0	0	0	0
	173	0	0	0.029	0	0	0	0	0	0	0	0
	177	0	0	0	0.022	0.017	0.013	0	0	0.005	0	0
	179	0	0.014	0	0	0	0.013	0.011	0	0.032	0	0
	183	0	0	0.029	0	0	0	0	0	0	0	0
	189	0	0	0	0.152	0.017	0.025	0	0	0	0	0
	193	0	0	0	0	0	0	0	0	0	0.009	0
	195	0	0.014	0	0	0	0	0	0	0	0.038	0
	197	0.022	0.014	0	0.022	0.052	0	0.011	0	0.005	0	0
	199	0.087	0	0	0	0	0	0	0	0	0	0
2N		92	70	34	46	58	80	88	78	188	106	50
Oneu2	200	0	0	0.143	0	0	0	0	0	0	0	0
	206	0	0	0.214	0.019	0	0	0	0	0	0	0
	208	0	0	0.107	0	0	0	0	0	0	0	0
	210	0.054	0.029	0	0	0	0	0.043	0	0.011	0.02	0
	212	0	0	0	0.115	0.071	0	0.053	0	0	0.01	0
	214	0.098	0.206	0.071	0.115	0.143	0.13	0.074	0.087	0.064	0.029	0
	216	0.12	0.044	0.214	0.096	0.095	0.019	0.032	0.162	0.096	0.059	0
	218	0	0.044	0.143	0.019	0	0.037	0.021	0	0.011	0	0
	220	0.011	0.015	0	0.058	0	0	0.043	0.013	0.053	0.118	0
	222	0.076	0.044	0	0.096	0.024	0.222	0.064	0.2	0.128	0.069	0
	224	0.152	0.074	0.071	0.096	0.19	0.167	0.181	0.1	0.106	0.186	0
	226	0.022	0.015	0	0	0	0	0.074	0.013	0.053	0	0
	228	0.359	0.412	0	0.173	0.262	0.278	0.213	0.287	0.372	0.304	0
	230	0.033	0.015	0	0.038	0.048	0.019	0.043	0.112	0.043	0.118	0
	232	0.076	0.088	0	0.173	0.167	0.093	0.138	0.025	0.043	0.088	0
	234	0	0	0	0	0	0.019	0.021	0	0.021	0	0
	238	0	0.015	0	0	0	0.019	0	0	0	0	0
	240	0	0	0.036	0	0	0	0	0	0	0	0
	244	0	0	0	0	0	0	0	0	0	0	0.095
	248	0	0	0	0	0	0	0	0	0	0	0.214
	250	0	0	0	0	0	0	0	0	0	0	0.548

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	252	0	0	0	0	0	0	0	0	0	0	0.024
	254	0	0	0	0	0	0	0	0	0	0	0.119
2N		92	68	28	52	42	54	94	80	94	102	42
Ots100	168	0	0.044	0	0	0	0	0	0	0	0	-
	172	0	0.029	0	0	0	0.079	0	0.077	0.023	0.102	-
	176	0.022	0.044	0	0	0.065	0.105	0.167	0.141	0.125	0.051	-
	182	0	0	0	0	0	0	0.011	0	0	0	-
	186	0	0	0	0.045	0	0	0	0	0	0	-
	188	0.022	0	0	0	0	0	0	0	0	0	-
	190	0.011	0	0	0	0	0	0	0.013	0	0	-
	192	0.065	0.015	0	0.068	0.087	0.237	0.078	0.051	0.045	0.02	-
	194	0	0	0.417	0	0.022	0	0	0	0	0	-
	196	0.033	0.015	0	0	0	0	0.144	0.013	0.057	0	-
	198	0	0.088	0	0.023	0	0	0.044	0	0.045	0.041	-
	202	0	0	0	0	0.022	0	0.044	0.013	0.011	0	-
	204	0.022	0.147	0	0.045	0.196	0	0.044	0.205	0.091	0.051	-
	206	0.087	0.029	0	0	0.022	0.026	0	0.038	0.023	0.01	-
	208	0.228	0.118	0	0.023	0.109	0.184	0.022	0.013	0.114	0.102	-
	210	0	0	0	0.045	0	0	0	0	0	0	-
	212	0	0.118	0	0.114	0.022	0	0.078	0.013	0.057	0.092	-
	214	0.054	0.103	0.125	0	0	0	0	0	0	0.01	-
	216	0	0	0.042	0.023	0	0	0	0	0.011	0	-
	220	0	0.015	0.125	0.023	0.043	0	0.022	0.013	0	0	-
	222	0.25	0.221	0.042	0.5	0.304	0.184	0.311	0.115	0.17	0.367	-
	224	0.207	0.015	0.208	0.091	0.087	0.158	0.033	0.282	0.17	0.143	-
	226	0	0	0	0	0	0.026	0	0.013	0.057	0.01	-
	232	0	0	0	0	0.022	0	0	0	0	0	-
	248	0	0	0.042	0	0	0	0	0	0	0	-
2N		92	68	24	44	46	38	90	78	88	98	-
Ssa14	131	0.043	0.015	0	0	0	0.05	0.021	0.038	0.012	0.029	0
	135	0	0	0	0.06	0.021	0	0	0	0.093	0	0
	137	0.087	0.103	0.059	0.1	0.063	0.05	0.16	0.125	0.093	0.067	0.333

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	139	0.587	0.662	0.824	0.68	0.75	0.8	0.67	0.65	0.547	0.817	0.667
	141	0.087	0	0	0	0	0	0	0	0.081	0	0
	143	0	0.044	0.059	0	0.083	0.017	0	0	0.012	0	0
	145	0	0	0	0.1	0.021	0.067	0	0	0.012	0	0
	147	0.174	0.162	0.059	0.04	0.063	0.017	0.149	0.188	0.128	0.087	0
	149	0.022	0.015	0	0.02	0	0	0	0	0.023	0	0
2N		92	68	34	50	48	60	94	80	86	104	48
Ots103	182	0.01	0.057	0.143	0	0	0.074	0.043	0	0.065	0.048	-
	186	0.979	0.929	0.571	0.981	0.962	0.926	0.894	0.934	0.913	0.942	-
	190	0.01	0.014	0.286	0.019	0.038	0	0.064	0.066	0.022	0.01	-
2N		96	70	28	52	52	54	94	76	92	104	-

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APPENDIX F. SCALE ANALYSIS RESULTS

Age notation follows that currently in use by the Washington Department of Fish and Wildlife. Smolt ages are the number of freshwater annuli. Adult ages are designated by the smolt age prior to the decimal, following the decimal, a "+" indicates partial years and separating post-smolt annuli, an "F" indicates a non-spawning annulus, and an "S" indicates a spawning annulus. An "R." indicates a partially regenerated scale for which a smolt age could not be assigned. Results are not given for fully regenerated scales or for ambiguous samples. Scale reading performed by John G. Sneva, Washington Department of Fish and Wildlife, Olympia, WA. Fork lenths are given in mm.

Stage	Date	Length	Creek	Sex	Age
smolt	3/22/95	232	Big Beef	unknown	2
smolt	3/23/95	255	Big Beef	unknown	2
smolt	3/24/95	204	Big Beef	unknown	2
smolt	3/24/95	215	Big Beef	unknown	2
smolt	3/24/95	232	Big Beef	unknown	2
smolt	3/27/95	210	Big Beef	unknown	2
smolt	3/29/95	252	Big Beef	unknown	2
smolt	3/29/95	277	Big Beef	unknown	2
smolt	3/29/95	235	Big Beef	unknown	2
smolt	3/31/95	151	Big Beef	unknown	2
smolt	3/29/94	228	Big Beef	unknown	3
smolt	4/1/94	165	Big Beef	unknown	2
smolt	4/3/94	170	Big Beef	unknown	3
smolt	4/3/94	190	Big Beef	unknown	3
smolt	4/3/94	195	Big Beef	unknown	3
smolt	4/4/94	165	Big Beef	unknown	3
smolt	4/4/94	162	Big Beef	unknown	2
smolt	4/5/94	136	Big Beef	unknown	2
smolt	4/5/94	176	Big Beef	unknown	2
smolt	4/6/94	210	Big Beef	unknown	3
smolt	4/9/94	141	Big Beef	unknown	2
smolt	4/11/94	161	Big Beef	unknown	3
smolt	4/12/94	208	Big Beef	unknown	3
smolt	4/13/94	186	Big Beef	unknown	2
smolt	4/13/94	196	Big Beef	unknown	2
smolt	4/13/94	200	Big Beef	unknown	2
smolt	4/14/94	186	Big Beef	unknown	1
smolt	4/15/94	170	Big Beef	unknown	2
smolt	4/15/94	173	Big Beef	unknown	2
smolt	4/15/94	235	Big Beef	unknown	2
smolt	4/17/94	162	Big Beef	unknown	2
smolt	4/17/94	158	Big Beef	unknown	2
smolt	4/18/94	144	Big Beef	unknown	2
smolt	4/18/94	162	Big Beef	unknown	2
smolt	4/18/94	189	Big Beef	unknown	2

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smolt	4/18/94	153	Big Beef	unknown	2
smolt	4/18/94	203	Big Beef	unknown	3
smolt	4/19/94	237	Big Beef	unknown	2
smolt	4/19/94	243	Big Beef	unknown	2
smolt	4/21/94	139	Big Beef	unknown	2
smolt	4/21/94	159	Big Beef	unknown	2
smolt	4/22/94	153	Big Beef	unknown	2
smolt	4/22/94	188	Big Beef	unknown	3
smolt	4/22/94	191	Big Beef	unknown	2
smolt	4/22/94	180	Big Beef	unknown	2
smolt	4/22/94	195	Big Beef	unknown	3
smolt	4/22/94	146	Big Beef	unknown	2
smolt	4/23/94	210	Big Beef	unknown	2
smolt	4/24/94	165	Big Beef	unknown	2
smolt	4/24/94	130	Big Beef	unknown	2
smolt	4/24/94	160	Big Beef	unknown	2
smolt	4/24/94	179	Big Beef	unknown	3
smolt	4/25/94	175	Big Beef	unknown	2
smolt	4/25/94	164	Big Beef	unknown	2
smolt	4/25/94	135	Big Beef	unknown	2
smolt	4/25/94	147	Big Beef	unknown	2
smolt	4/25/94	214	Big Beef	unknown	3
smolt	4/25/94	144	Big Beef	unknown	2
smolt	4/25/94	181	Big Beef	unknown	2
smolt	4/25/94	183	Big Beef	unknown	2
smolt	4/25/94	207	Big Beef	unknown	2
smolt	4/26/94	129	Big Beef	unknown	1
smolt	4/26/94	160	Big Beef	unknown	2
smolt	4/27/94	125	Big Beef	unknown	2
smolt	4/27/94	145	Big Beef	unknown	2
smolt	4/27/94	150	Big Beef	unknown	2
smolt	4/27/94	172	Big Beef	unknown	3
smolt	4/27/94	139	Big Beef	unknown	2
smolt	4/27/94	173	Big Beef	unknown	3
smolt	4/27/94	196	Big Beef	unknown	2
smolt	4/28/94	153	Big Beef	unknown	2
smolt	4/28/94	144	Big Beef	unknown	2
smolt	4/28/94	138	Big Beef	unknown	2
smolt	4/28/94	161	Big Beef	unknown	2
smolt	4/28/94	141	Big Beef	unknown	2
smolt	4/28/94	139	Big Beef	unknown	2
smolt	4/28/94	161	Big Beef	unknown	2
smolt	4/28/94	136	Big Beef	unknown	2
smolt	4/28/94	139	Big Beef	unknown	2
smolt	4/28/94	156	Big Beef	unknown	2
smolt	4/29/94	149	Big Beef	unknown	2
smolt	4/29/94	130	Big Beef	unknown	2
smolt	4/29/94	158	Big Beef	unknown	2
smolt	4/29/94	124	Big Beef	unknown	2

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smolt	4/29/94	144	Big Beef	unknown	2
smolt	4/29/94	136	Big Beef	unknown	2
smolt	4/29/94	194	Big Beef	unknown	3
smolt	4/30/94	141	Big Beef	unknown	2
smolt	4/30/94	166	Big Beef	unknown	3
smolt	4/30/94	160	Big Beef	unknown	3
smolt	4/30/94	144	Big Beef	unknown	2
smolt	4/30/94	124	Big Beef	unknown	2
smolt	4/30/94	152	Big Beef	unknown	3
smolt	4/30/94	218	Big Beef	unknown	3
smolt	5/1/94	134	Big Beef	unknown	2
smolt	5/1/94	148	Big Beef	unknown	2
smolt	5/1/94	183	Big Beef	unknown	2
smolt	5/1/94	136	Big Beef	unknown	2
smolt	5/1/94	149	Big Beef	unknown	2
smolt	5/1/94	152	Big Beef	unknown	2
smolt	5/2/94	136	Big Beef	unknown	2
smolt	5/2/94	155	Big Beef	unknown	2
smolt	5/2/94	162	Big Beef	unknown	2
smolt	5/2/94	137	Big Beef	unknown	2
smolt	5/3/94	139	Big Beef	unknown	2
smolt	5/3/94	153	Big Beef	unknown	2
smolt	5/3/94	137	Big Beef	unknown	2
smolt	5/3/94	156	Big Beef	unknown	2
smolt	5/3/94	144	Big Beef	unknown	2
smolt	5/4/94	130	Big Beef	unknown	2
smolt	5/4/94	140	Big Beef	unknown	2
smolt	5/4/94	137	Big Beef	unknown	2
smolt	5/4/94	110	Big Beef	unknown	2
smolt	5/4/94	146	Big Beef	unknown	2
smolt	5/4/94	137	Big Beef	unknown	2
smolt	5/4/94	165	Big Beef	unknown	2
smolt	5/4/94	134	Big Beef	unknown	2
smolt	5/4/94	102	Big Beef	unknown	1
smolt	5/4/94	122	Big Beef	unknown	2
smolt	5/4/94	152	Big Beef	unknown	2
smolt	5/4/94	125	Big Beef	unknown	2
smolt	5/4/94	125	Big Beef	unknown	2
smolt	5/4/94	156	Big Beef	unknown	2
smolt	5/4/94	144	Big Beef	unknown	2
smolt	5/4/94	138	Big Beef	unknown	2
smolt	5/4/94	142	Big Beef	unknown	2
smolt	5/5/94	132	Big Beef	unknown	2
smolt	5/5/94	164	Big Beef	unknown	2
smolt	5/5/94	137	Big Beef	unknown	2
smolt	5/5/94	158	Big Beef	unknown	2
smolt	5/5/94	147	Big Beef	unknown	2
smolt	5/8/94	227	Big Beef	unknown	3
smolt	5/8/94	208	Big Beef	unknown	2

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smolt	4/2/95	208	Big Beef	unknown	2
smolt	4/2/95	176	Big Beef	unknown	2
smolt	4/3/95	168	Big Beef	unknown	3
smolt	4/4/95	147	Big Beef	unknown	2
smolt	4/5/95	191	Big Beef	unknown	3
smolt	4/5/95	188	Big Beef	unknown	3
smolt	4/5/95	247	Big Beef	unknown	2
smolt	4/5/95	207	Big Beef	unknown	2
smolt	4/6/95	197	Big Beef	unknown	2
smolt	4/6/95	176	Big Beef	unknown	2
smolt	4/6/95	215	Big Beef	unknown	2
smolt	4/6/95	164	Big Beef	unknown	2
smolt	4/6/95	190	Big Beef	unknown	3
smolt	4/6/95	199	Big Beef	unknown	2
smolt	4/6/95	169	Big Beef	unknown	3
smolt	4/6/95	143	Big Beef	unknown	3
smolt	4/7/95	215	Big Beef	unknown	3
smolt	4/7/95	266	Big Beef	unknown	3
smolt	4/7/95	132	Big Beef	unknown	2
smolt	4/9/95	172	Big Beef	unknown	2
smolt	4/9/95	243	Big Beef	unknown	2
smolt	4/9/95	156	Big Beef	unknown	2
smolt	4/9/95	121	Big Beef	unknown	2
smolt	4/9/95	147	Big Beef	unknown	2
smolt	4/9/95	132	Big Beef	unknown	2
smolt	4/10/95	153	Big Beef	unknown	2
smolt	4/10/95	204	Big Beef	unknown	2
smolt	4/10/95	215	Big Beef	unknown	2
smolt	4/10/95	139	Big Beef	unknown	2
smolt	4/10/95	229	Big Beef	unknown	2
smolt	4/10/95	106	Big Beef	unknown	1
smolt	4/11/95	225	Big Beef	unknown	2
smolt	4/11/95	247	Big Beef	unknown	2
smolt	4/11/95	226	Big Beef	unknown	2
smolt	4/11/95	202	Big Beef	unknown	2
smolt	4/11/95	217	Big Beef	unknown	2
smolt	4/12/95	158	Big Beef	unknown	2
smolt	4/12/95	221	Big Beef	unknown	2
smolt	4/12/95	237	Big Beef	unknown	2
smolt	4/12/95	233	Big Beef	unknown	2
smolt	4/12/95	208	Big Beef	unknown	2
smolt	4/12/95	162	Big Beef	unknown	3
smolt	4/13/95	216	Big Beef	unknown	2
smolt	4/13/95	147	Big Beef	unknown	2
smolt	4/13/95	149	Big Beef	unknown	2
smolt	4/13/95	137	Big Beef	unknown	2
smolt	4/14/95	126	Big Beef	unknown	2
smolt	4/14/95	139	Big Beef	unknown	2
smolt	4/14/95	218	Big Beef	unknown	2

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smolt	4/14/95	163	Big Beef	unknown	2
smolt	4/14/95	199	Big Beef	unknown	2
smolt	4/14/95	227	Big Beef	unknown	2
smolt	4/14/95	179	Big Beef	unknown	2
smolt	4/14/95	180	Big Beef	unknown	3
smolt	4/14/95	208	Big Beef	unknown	3
smolt	4/17/95	142	Big Beef	unknown	2
smolt	4/17/95	170	Big Beef	unknown	2
smolt	4/17/95	185	Big Beef	unknown	2
smolt	4/17/95	217	Big Beef	unknown	2
smolt	4/17/95	127	Big Beef	unknown	2
smolt	4/19/95	220	Big Beef	unknown	2
smolt	4/19/95	210	Big Beef	unknown	2
smolt	4/20/95	235	Big Beef	unknown	2
smolt	4/21/95	115	Big Beef	unknown	2
smolt	4/24/95	111	Big Beef	unknown	1
smolt	4/30/95	245	Big Beef	unknown	3
smolt	5/17/95	251	Big Beef	unknown	2
smolt	5/6/94	135	Stavis	unknown	2
smolt	5/6/94	122	Stavis	unknown	2
smolt	5/6/94	161	Stavis	unknown	3
smolt	5/6/94	112	Stavis	unknown	2
smolt	5/6/94	129	Stavis	unknown	2
smolt	5/6/94	108	Stavis	unknown	2
smolt	5/6/94	150	Stavis	unknown	2
smolt	5/6/94	121	Stavis	unknown	2
smolt	5/6/94	140	Stavis	unknown	2
smolt	5/6/94	152	Stavis	unknown	2
smolt	5/23/94	134	Stavis	unknown	2
smolt	5/23/94	135	Stavis	unknown	2
smolt	5/23/94	140	Stavis	unknown	2
smolt	5/23/94	128	Stavis	unknown	2
smolt	5/23/94	135	Stavis	unknown	2
smolt	5/23/94	125	Stavis	unknown	2
smolt	5/23/94	127	Stavis	unknown	2
smolt	5/24/94	167	Stavis	unknown	2
smolt	5/24/94	155	Stavis	unknown	2
smolt	5/24/94	137	Stavis	unknown	2
smolt	5/24/94	128	Stavis	unknown	2
smolt	5/24/94	137	Stavis	unknown	2
smolt	5/27/94	125	Stavis	unknown	2
smolt	5/27/94	127	Stavis	unknown	2
smolt	5/27/94	123	Stavis	unknown	2
smolt	5/27/94	134	Stavis	unknown	2
smolt	4/11/95	132	Stavis	unknown	2
smolt	4/15/95	200	Stavis	unknown	4
smolt	4/17/95	153	Stavis	unknown	2
smolt	4/17/95	122	Stavis	unknown	2
smolt	4/17/95	132	Stavis	unknown	2

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smolt	4/17/95	96	Stavis	unknown	1
smolt	4/17/95	132	Stavis	unknown	2
smolt	4/17/95	152	Stavis	unknown	2
smolt	5/1/95	243	Stavis	unknown	4
adult	12/29/93	363	Big Beef	Female	2.+
adult	12/30/93	335	Big Beef	Male	2.+
adult	12/31/93	420	Big Beef	Male	3.+
adult	1/3/94	315	Big Beef	Male	R.+
adult	1/3/94	350	Big Beef	Male	2.+
adult	1/11/94	350	Big Beef	Female	R.+
adult	1/11/94	325	Big Beef	Male	R.+
adult	1/11/94	440	Big Beef	Female	3.+S+
adult	1/12/94	337	Big Beef	Female	R.+
adult	1/12/94	350	Big Beef	Male	R.+
adult	1/12/94	351	Big Beef	Female	2.+
adult	1/12/94	345	Big Beef	Female	R.+
adult	1/13/94	341	Big Beef	Female	R.+
adult	1/13/94	367	Big Beef	Male	R.+
adult	1/13/94	351	Big Beef	Male	R.+
adult	1/15/94	368	Big Beef	Female	3.+
adult	1/16/94	377	Big Beef	Female	2.+
adult	1/16/94	387	Big Beef	Female	2.+S+
adult	1/18/94	355	Big Beef	Female	R.+
adult	1/23/94	331	Big Beef	Male	2.+
adult	1/23/94	425	Big Beef	Female	R.+F+
adult	1/24/94	356	Big Beef	Female	2.+
adult	1/24/94	372	Big Beef	Male	R.+
adult	1/24/94	222	Big Beef	Male	2.+
adult	1/24/94	312	Big Beef	Female	R.+
adult	1/24/94	310	Big Beef	Female	3.+
adult	1/27/94	360	Big Beef	Female	R.+
adult	1/27/94	401	Big Beef	Female	R.+S+
adult	1/29/94	378	Big Beef	Male	R.+
adult	2/6/94	226	Big Beef	Male	R.+
adult	2/6/94	320	Big Beef	Female	3.+
adult	2/11/94	372	Big Beef	Male	R.+
adult	2/18/94	362	Big Beef	Female	R.+
adult	2/22/94	335	Big Beef	Male	2.+
adult	2/25/94	323	Big Beef	Female	R.+
adult	2/26/94	361	Big Beef	Male	R.+
adult	2/26/94	375	Big Beef	Female	3.+
adult	2/26/94	337	Big Beef	Female	2.+
adult	2/26/94	326	Big Beef	Female	2.+
adult	2/26/94	365	Big Beef	Female	2.+
adult	2/26/94	341	Big Beef	Female	R.+
adult	2/26/94	358	Big Beef	Male	2.+
adult	2/27/94	387	Big Beef	Male	R.+
adult	2/28/94	362	Big Beef	Female	R.+
adult	2/28/94	348	Big Beef	Female	R.+

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adult	2/28/94	384	Big Beef	Female	R.+
adult	3/1/94	377	Big Beef	Female	R.+
adult	3/2/94	382	Big Beef	Male	R.+
adult	3/2/94	451	Big Beef	Female	R.+S+
adult	3/2/94	320	Big Beef	Female	2.+
adult	3/2/94	340	Big Beef	Female	2.+
adult	3/2/94	220	Big Beef	Male	2.+
adult	3/2/94	359	Big Beef	Female	R.+
adult	3/3/94	364	Big Beef	Female	R.+
adult	3/4/94	335	Big Beef	Female	2.+
adult	3/6/94	341	Big Beef	Female	R.+
adult	3/10/94	353	Big Beef	Female	2.+
adult	3/10/94	400	Big Beef	Male	2.+
adult	3/11/94	331	Big Beef	Female	R.+
adult	3/14/94	369	Big Beef	Male	3.+
adult	3/14/94	402	Big Beef	Female	R.+
adult	3/14/94	366	Big Beef	Female	2.+
adult	3/14/94	333	Big Beef	Female	R.+
adult	3/16/94	331	Big Beef	Female	2.+
adult	3/17/94	346	Big Beef	Female	R.+
adult	3/18/94	342	Big Beef	Female	R.+
adult	3/20/94	374	Big Beef	Female	R.+
adult	3/30/94	337	Big Beef	Female	R.+
adult	4/3/94	360	Big Beef	Female	R.+
adult	4/7/94	340	Big Beef	Female	R.+
adult	4/11/94	282	Big Beef	Male	R.+
adult	4/11/94	354	Big Beef	Female	R.+
adult	4/11/94	351	Big Beef	Male?	2.+
adult	4/17/94	379	Big Beef	Male	R.+
adult	4/25/94	210	Big Beef	Female	R.+
adult	4/29/94	392	Big Beef	Female	R.+S+
adult	10/27/94	414	Big Beef	Female	R.+S+
adult	11/1/94	395	Big Beef	Female	R.+S+
adult	11/6/94	294	Big Beef	Male	R.+
adult	11/6/94	209	Big Beef	Male	R.+
adult	11/6/94	312	Big Beef	Female	2.+F+
adult	11/11/94	336	Big Beef	Female	R.+
adult	12/2/94	425	Big Beef	Female	R.+F+
adult	12/15/94	357	Big Beef	Male	R.+
adult	1/8/95	320	Big Beef	Male	R.+
adult	1/17/95	352	Big Beef	Female	R.+
adult	1/28/95	418	Big Beef	Female	R.+S+
adult	1/29/95	450	Big Beef	Female	R.+F+
adult	1/29/95	332	Big Beef	Male	R.+
adult	1/30/95	334	Big Beef	Male	R.+S+
adult	2/2/95	365	Big Beef	Female	2.+F+
adult	2/3/95	324	Big Beef	Male	R.+
adult	2/3/95	351	Big Beef	Female	3.+
adult	2/3/95	337	Big Beef	Female	3.+

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adult	2/4/95	454	Big Beef	Female	R.+F+
adult	2/5/95	361	Big Beef	Female	2.+
adult	2/5/95	329	Big Beef	Male	2.+
adult	2/5/95	381	Big Beef	Female	2.+F+
adult	2/5/95	366	Big Beef	Male	R.+
adult	2/5/95	366	Big Beef	Female	R.+
adult	2/5/95	379	Big Beef	Female	R.+S+
adult	2/6/95	328	Big Beef	Female	R.+
adult	2/6/95	443	Big Beef	Female	2.+F+
adult	2/6/95	349	Big Beef	Female	2.+
adult	2/6/95	421	Big Beef	Female	R.+
adult	2/6/95	400	Big Beef	Female	R.+
adult	2/6/95	409	Big Beef	Female	R.+F+
adult	2/8/95	311	Big Beef	Female	R.+
adult	2/21/95	430	Big Beef	Female	R.+S+
adult	2/26/95	353	Big Beef	Female	2.+
adult	3/7/95	177	Big Beef	Male	1.+
adult	3/9/95	376	Big Beef	Male	3.+
adult	3/9/95	440	Big Beef	Female	R.+S+
adult	3/14/95	400	Big Beef	Male	R.+
adult	3/14/95	370	Big Beef	Female	3.+
adult	3/18/95	295	Big Beef	Male	R.+
adult	3/18/95	340	Big Beef	Male	2.+
adult	3/20/95	409	Big Beef	Female	R.+F+
adult	3/22/95	348	Big Beef	Male	2.+F+
adult	3/22/95	365	Big Beef	Female	2.+
adult	3/28/95	392	Big Beef	Male	2.+
adult	3/29/95	395	Big Beef	Male	R.+S+S+
adult	3/29/95	348	Big Beef	Female	2.+
adult	3/29/95	313	Big Beef	Female	R.+F+
adult	3/29/95	383	Big Beef	Female	R.+
adult	4/1/95	355	Big Beef	Female	R.+S+
adult	4/3/95		Big Beef	Female	2.+S+
adult	4/4/95	364	Big Beef	Male	4.+
adult	4/5/95	375	Big Beef	Male	R.+
adult	4/7/95	335	Big Beef	Female	2.+
adult	4/7/95	365	Big Beef	Female	3.+
adult	4/7/95	358	Big Beef	Female	2.+
adult	4/7/95	356	Big Beef	Male	2.+
adult	4/9/95	244	Big Beef	Male	3.+
adult	4/10/95	344	Big Beef	Female	2.+
adult	4/10/95	370	Big Beef	Female	2.+
adult	4/10/95	338	Big Beef	Female	2.+
adult	4/10/95	350	Big Beef	Female	R.+
adult	4/11/95	319	Big Beef	Female	2.+
adult	4/19/95	320	Big Beef	Female	2.+
adult	4/23/95	394	Big Beef	Female	R.+S+
adult	12/19/95	352	Big Beef	Male	2.+
adult	12/17/95	366	Big Beef	Male	2.+

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adult	1/3/96	347	Big Beef	Female	2.+
adult	3/30/96	361	Big Beef	Female	2.+
adult	3/13/96	320	Big Beef	Male	R.+
adult	12/1/95	321	Big Beef	Female	2.+
adult	11/11/95	280	Big Beef	Male	2.+
adult	4/7/96	394	Big Beef	Female	2.+S+
adult	1/15/96	473	Big Beef	Male	R.+S+
adult	11/1/95	350	Big Beef	Female	R.+S+
adult	11/12/95	267	Big Beef	Male	3.+
adult	11/12/95	386	Big Beef	Male	3.+
adult	11/22/95	330	Big Beef	Female	R.+
adult	11/23/95	332	Big Beef	Female	2.+
adult	11/25/95	451	Big Beef	Female	2.+F+
adult	11/25/95	326	Big Beef	Male	R.+
adult	11/25/95	350	Big Beef	Male	R.+
adult	11/25/95	352	Big Beef	Male	3.+
adult	4/22/96	344	Big Beef	Male	3.+
adult	11/30/95	359	Big Beef	Female	2.+
adult	11/30/95	169	Big Beef	Female	R.+
adult	11/30/95	375	Big Beef	Male	R.+
adult	12/1/95	365	Big Beef	Female	2.+
adult	2/1/96	328	Big Beef	Male	R.+
adult	12/20/95	272	Big Beef	Male	1.+
adult	12/21/95	315	Big Beef	Female	R.+
adult	12/21/95	310	Big Beef	Male	2.+
adult	1/3/96	333	Big Beef	Female	2.+
adult	1/3/96	396	Big Beef	Female	3.+
adult	1/3/96	378	Big Beef	Male	2.+F+S+
adult	1/3/96	330	Big Beef	Male	R.+
adult	1/3/96	347	Big Beef	Male	2.+
adult	1/6/96	330	Big Beef	Male	2.+
adult	1/8/96	350	Big Beef	Female	R.+
adult	1/9/96	428	Big Beef	Female	2.+F+
adult	1/13/96	360	Big Beef	Male	2.+
adult	1/14/96	364	Big Beef	Female	2.+
adult	1/14/96	366	Big Beef	Female	2.+
adult	1/15/96	356	Big Beef	Female	R.+
adult	1/15/96	346	Big Beef	Male	R.+
adult	1/15/96	205	Big Beef	Male	2.+
adult	1/15/96	317	Big Beef	Female	R.+
adult	1/15/96	359	Big Beef	Male	2.+
adult	1/15/96	332	Big Beef	Male	2.+
adult	1/16/96	328	Big Beef	Female	2.+
adult	2/1/96	384	Big Beef	Female	2.+
adult	2/1/96	306	Big Beef	Male	2.+
adult	2/1/96	312	Big Beef	Female	2.+
adult	2/1/96	439	Big Beef	Female	R.+S+
adult	2/1/96	365	Big Beef	Female	2.+
adult	2/1/96	305	Big Beef	Female	R.+

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adult	2/1/96	362	Big Beef	Male	R.+
adult	2/7/96	332	Big Beef	Female	2.+
adult	2/7/96	409	Big Beef	Male	R.+
adult	2/9/96	391	Big Beef	Female	2.+
adult	2/19/96	410	Big Beef	Female	R.+
adult	3/20/96	338	Big Beef	Female	2.+
adult	1/15/96	422	Big Beef	Male	2.+S+
adult	3/28/96	355	Big Beef	Female	R.+
adult	12/10/95	425	Big Beef	Female	2.+S+
adult	3/31/96	284	Big Beef	Male	R.+
adult	4/7/96	362	Big Beef	Male	2.+
adult	4/2/96	352	Big Beef	Female	2.+
adult	4/11/96	325	Big Beef	Female	2.+
adult	2/1/96	348	Big Beef	Female	R.+
adult	1/3/96	423	Big Beef	Female	2.+F+
adult	3/4/96	380	Big Beef	Female	R.+S+
adult	2/21/96	485	Big Beef	Female	R.+S+s+
adult	1/11/96	430	Big Beef	Female	R.+S+s+
adult	2/17/96	452	Big Beef	Female	R.+F+S+S+
adult	1/15/96	443	Big Beef	Male	2.+S+
adult	1/15/96	417	Big Beef	Female	2.+S+
adult	1/3/96	434	Big Beef	Female	R.+F+
adult	3/18/96	458	Big Beef	Female	2.+F+S+
adult	1/7/96	380	Big Beef	Female	R.+S+
adult	1/17/96	352	Big Beef	Male	3.+
adult	4/7/96	386	Big Beef	Female	2.+

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APPENDIX G. DATA FOR RECAPTURED COASTAL CUTTHROAT TROUT IN BIG BEEF CREEK FROM 1993-1996.

Creek	Stage	Date	Trap	Weight	Length	VI tag	#*	Sex
Big Beef	smolt	4/13/95	down	79	201	A17	1	1
Big Beef	adult	12/19/95	up	424	352	A17	2	1
Big Beef	smolt	4/19/95	down	90	215	A45	1	1
Big Beef	adult	12/17/95	up	466	366	A45	2	1
Big Beef	smolt	4/19/95	down	80	197	A48	1	2
Big Beef	adult	1/3/96	up	393	347	A48	2	2
Big Beef	adult	1/15/96	up	479	350	A49	1	2
Big Beef	adult	4/25/96	down	370	339	A49	2	2
Big Beef	adult	1/15/96	up	563	367	A50	1	2
Big Beef	adult	4/17/96	down	315	365	A50	2	2
Big Beef	smolt	4/20/95	down	35	159	A52	1	2
Big Beef	adult	3/30/96	up	506	361	A52	2	2
Big Beef	adult	4/12/96	down	420	356	A52	3	2
Big Beef	smolt	4/21/95	down	153	249	A62	1	2
Big Beef	adult	12/2/95	up	506	363	A62	2	2
Big Beef	smolt	4/21/95	down	37	160	A72	1	1
Big Beef	adult	11/1/95	up	438	338	A72	2	1
Big Beef	adult	3/13/96	down	268	320	A72	3	1
Big Beef	smolt	4/22/95	down	75	200	A78	1	2
Big Beef	adult	12/1/95	up	368	321	A78	2	2
Big Beef	adult	4/19/96	down	290	315	A78	3	2
Big Beef	smolt	4/22/95	down	49	173	A85	1	1
Big Beef	adult	4/13/96	down	na	345	A85	2	1
Stavis	smolt	5/16/95	down	na	168	AF9	1	1
Big Beef	adult	11/11/95	up	212	280	AF9	2	1
Big Beef	smolt	4/15/94	down	88	210	BA4	1	2
Big Beef	adult	1/29/95	up	500	354	BA4	2	2
Big Beef	adult	4/19/95	down	375	351	BA4	3	2
Big Beef	adult	11/12/95	up	782	408	BA4	4	2
Big Beef	adult	2/20/96	up	862	418	BA4	5	2
Big Beef	adult	4/7/96	down	598	394	BA4	6	2
Big Beef	smolt	4/15/94	down	82	205	BA8	1	2
Big Beef	adult	3/14/95	up	509	370	BA8	2	2
Big Beef	smolt	4/15/94	down	118	229	BA9	1	1
Big Beef	adult	3/31/95	down	549	400	BA9	2	1
Big Beef	adult	1/15/96	up	1174	473	BA9	3	1
Big Beef	smolt	4/18/94	down	61	189	BE2	1	
Big Beef	adult	4/30/95	down	na	352	BE2	2	
Big Beef	smolt	4/19/94	down	123	228	BH0	1	
Big Beef	adult	3/9/95	up	598	376	BH0	2	1
Big Beef	adult	4/5/95	down	503	370	BH0	3	1
Big Beef	adult	4/7/94	down	316	340	C15	1	2
Big Beef	adult	11/1/94	up	620	395	C15	2	1
Big Beef	smolt	4/10/94	down	67	190	C46	1	

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Big Beef	adult	12/3/95	up	675	385	C46	2	2
Big Beef	adult	4/11/94	down	181	282	C48	1	1
Big Beef	adult	1/30/95	up	410	334	C48	2	1
Big Beef	adult	3/29/95	down	279	327	C48	3	1
Big Beef	adult	4/11/94	down	267	321	C49	1	2
Big Beef	adult	2/5/95	up	596	379	C49	2	2
Big Beef	adult	4/8/95	down	na	na	C49	3	2
Big Beef	adult	4/4/94	up	353	320	C68	1	2
Big Beef	adult	4/26/94	down	SB	320	C68	2	2
Big Beef	adult	5/5/94	down	242	320	C68	3	2
Big Beef	adult	4/7/95	down	403	364	C68	4	2
Big Beef	adult	3/30/94	down	374	337	C87	1	2
Big Beef	adult	3/9/95	up	972	440	C87	2	2
Big Beef	smolt	4/14/94	down	78	198	CN0	1	
Big Beef	adult	1/8/96	up	1003	444	CN0	2	2
Big Beef	smolt	4/14/94	down	47	165	CN4	1	
Big Beef	adult	2/6/95	up	494	349	CN4	2	2
Big Beef	adult	4/16/95	down		355	CN4	3	
Big Beef	smolt	4/25/95	down	52	176	D72	1	
Big Beef	adult	4/1/96	up	473	355	D72	2	2
Big Beef	adult	1/27/94	up	528	360	DA0	1	2
Big Beef	adult	4/22/94	down	421	359	DA0	2	2
Big Beef	adult	1/25/94	up	569	367	DA1	1	2
Big Beef	adult	2/16/94	down	555	367	DA1	2	2
Big Beef	adult	3/12/94	up	523	367	DA1	3	2
Big Beef	adult	4/11/94	down	463	369	DA1	4	2
Big Beef	adult	1/23/94	up	782	425	DB1	1	2
Big Beef	adult	2/22/94	down	618	423	DB1	2	2
Big Beef	adult	1/22/94	up	832	412	DB2	1	2
Big Beef	adult	2/22/94	down	648	404	DB2	2	2
Big Beef	adult	1/16/94	up	593	377	DB6	1	2
Big Beef	adult	4/7/94	down	480	375	DB6	2	2
Big Beef	adult	1/16/94	up	664	387	DB7	1	2
Big Beef	adult	3/2/94	down	560	382	DB7	2	1
Big Beef	adult	1/15/94	up	544	368	DB8	1	2
Big Beef	adult	1/23/94	down	449	365	DB8	2	2
Big Beef	adult	1/13/94	up	520	367	DC3	1	1
Big Beef	adult	3/20/94	down	388	365	DC3	2	1
Big Beef	adult	1/13/94	up	450	351	DC5	1	1
Big Beef	adult	4/7/94	down	336	345	DC5	2	1
Big Beef	adult	1/12/94	up	1042	438	DC8	1	1
Big Beef	adult	3/18/94	down	808	430	DC8	2	1
Big Beef	adult	1/3/94	up	335	315	DD9	1	1
Big Beef	adult	4/8/94	down	224	306	DD9	2	1
Big Beef	adult	1/3/94	up	525	337	DE0	1	1
Big Beef	adult	3/30/94	down	388	350	DE0	2	1
Big Beef	adult	1/1/94	up	551	363	DE2	1	2
Big Beef	adult	1/23/94	down	540	378	DE2	2	2
Big Beef	adult	12/30/93	up	378	335	DE8	1	1
Big Beef	adult	2/22/94	down	386	335	DE8	2	1
Big Beef	adult	3/1/94	up	na	na	DE8	3	
Big Beef	adult	4/20/94	down	294	330	DE8	4	1

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Big Beef	smolt	5/25/95	down	31	149	E23	1	
Big Beef	adult	4/10/96	down	318	285	E23	2	1
Stavis	smolt	5/25/95	down	na	303	E33	1	2
Big Beef	adult	12/17/95	up	481	348	E33	2	2
Big Beef	adult	11/15/95	up	713	395	E73	1	1
Big Beef	adult	3/25/96	down	412	391	E73	2	1
Big Beef	adult	11/22/95	up	434	330	E75	1	2
Big Beef	adult	4/6/96	down	312	334	E75	2	2
Big Beef	adult	11/23/95	up	398	332	E76	1	2
Big Beef	adult	5/7/96	down	349	336	E76	2	2
Big Beef	adult	11/25/95	up	462	350	E80	1	1
Big Beef	adult	4/13/96	down	na	339	E80	2	1
Big Beef	adult	11/29/95	up	651	402	E83	1	2
Big Beef	adult	12/1/95	up	662	395	E83	2	2
Big Beef	adult	11/29/95	up	373	329	E84	1	1
Big Beef	adult	4/22/96	down	322	344	E84	2	1
Big Beef	smolt	5/4/95	down	28	139	EE4	1	
Big Beef	adult	4/30/96	down	na	360	EE4	2	2
Stavis	smolt	5/8/95	down	na	171	EH3	1	
Big Beef	adult	2/1/96	up	445	328	EH3	2	1
Stavis	smolt	4/7/95	down	na	141	F04	1	
Big Beef	adult	5/16/96	down	230	218	F04	2	1
Big Beef	adult	12/21/95	up	683	379	FA9	1	2
Big Beef	adult	3/25/96	down	380	378	FA9	2	2
Big Beef	adult	12/21/95	up	366	310	FB0	1	1
Big Beef	adult	4/17/96	down	na	na	FB0	2	1
Big Beef	adult	1/3/96	up	395	330	FB6	1	1
Big Beef	adult	4/8/96	down	365	318	FB6	2	1
Big Beef	adult	1/14/96	up	581	384	FC9	1	2
Big Beef	adult	4/13/96	down	na	376	FC9	2	2
Big Beef	adult	1/15/96	up	509	356	FD2	1	2
Big Beef	adult	4/12/96	down	400	371	FD2	2	2
Big Beef	adult	1/15/96	up	490	359	FD7	1	1
Big Beef	adult	4/12/96	down	426	350	FD7	2	1
Big Beef	adult	2/1/96	up	1073	439	FF0	1	2
Big Beef	adult	2/15/96	up	1050	445	FF0	2	2
Big Beef	adult	2/1/96	up	521	365	FF2	1	2
Big Beef	adult	4/3/96	down	388	358	FF2	2	2
Big Beef	adult	2/1/96	up	294	305	FF3	1	2
Big Beef	adult	4/2/96	down	328	296	FF3	2	2
Big Beef	adult	2/1/96	up	370	328	FF4	1	1
Big Beef	adult	4/16/96	down	283	321	FF4	2	1
Big Beef	adult	2/7/96	up	709	409	FF8	1	1
Big Beef	adult	4/2/96	down	461	395	FF8	2	1
Big Beef	adult	2/20/96	up	700	390	FH2	1	2
Big Beef	adult	3/25/96	down	429	387	FH2	2	2
Big Beef	adult	3/20/96	up	413	338	FJ0	1	2
Big Beef	adult	4/30/96	down	298	335	FJ0	2	2
Big Beef	adult	3/18/95	down	229	295	H04	1	1
Big Beef	adult	1/3/96	up	642	390	H04	2	1
Big Beef	adult	1/15/96	up	870	422	H05	3	1
Big Beef	smolt	3/24/95	down	89	215	H12	1	x

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Big Beef	adult	4/8/96	down	362	312	H12	2	2
Big Beef	smolt	3/27/95	down	61	192	H15	1	x
Big Beef	adult	3/28/96	down	412	355	H15	2	2
Big Beef	adult	3/29/95	down	329	348	H21	1	2
Big Beef	adult	12/10/95	up	768	425	H21	2	2
Big Beef	adult	3/31/95	down	200	291	H28	1	1
Big Beef	adult	11/11/95	up	623	383	H28	2	1
Big Beef	smolt	4/3/95	down	43	168	H36	1	x
Big Beef	adult	3/20/96	up	562	369	H36	2	2
Big Beef	smolt	4/4/95	down	255	285	H37	1	x
Big Beef	adult	4/6/96	down	461	372	H37	2	2
Big Beef	adult	4/1/95	down	554	395	H48	1	2
Big Beef	adult	2/1/96	up	883	434	H48	2	2
Big Beef	adult	4/1/95	down	240	317	H49	1	1
Big Beef	adult	12/8/95	up	633	382	H49	2	1
Big Beef	adult	4/7/96	down	na	374	H49	3	1
Big Beef	smolt	4/6/95	down	52	176	H53	1	x
Big Beef	adult	3/31/96	down	481	284	H53	2	1
Big Beef	smolt	4/6/95	down	36	164	H56	1	x
Big Beef	adult	4/7/96	down	409	362	H56	2	1
Big Beef	adult	4/6/95	down	416	361	H64	1	2
Big Beef	adult	12/2/95	up	990	429	H64	2	2
Big Beef	smolt	4/7/95	down	105	220	H66	1	x
Big Beef	adult	12/19/95	up	563	362	H66	2	2
Big Beef	adult	4/9/95	down	135	244	H83	1	1
Big Beef	adult	11/8/95	up	na	383	H83	2	1
Big Beef	smolt	4/9/95	down	89	197	H87	1	x
Big Beef	adult	11/29/95	up	474	342	H87	2	2
Big Beef	adult	4/10/95	up	309	347	H95	1	1
Big Beef	adult	4/11/95	down	312	na	H95	2	1
Big Beef	adult	4/10/95	up	383	344	H96	1	2
Big Beef	adult	4/11/95	down	380	na	H96	2	2
Big Beef	smolt	4/28/95	down	21	131	HB9	1	
Big Beef	adult	2/1/96	up	345	310	HB9	2	2
Big Beef	smolt	5/1/95	down	na	218	HL4	1	
Big Beef	adult	4/2/96	up	370	352	HL4	2	2
Big Beef	smolt	5/8/94	down	30	135	J19	1	
Big Beef	adult	1/10/96	up	473	344	J19	2	2
Big Beef	smolt	5/8/94	down	20	122	J24	1	
Big Beef	adult	2/1/96	up	483	348	J24	2	2
Big Beef	smolt	5/4/94	down	32	156	L36	1	
Big Beef	adult	1/3/96	up	824	423	L36	2	2
Big Beef	adult	2/1/96	up	624	415	L36	3	2
Big Beef	adult	4/11/95	down	290	319	M60	1	2
Big Beef	adult	3/4/96	down	495	380	M60	2	2
Big Beef	smolt	4/11/95	down	121	226	M63	1	x
Big Beef	adult	11/30/95	up	480	343	M63	2	2
Big Beef	smolt	4/26/95	down	49	175	R12	1	x
Big Beef	adult	10/26/95	up	na	310	R12	2	2
Little Anderson	adult	4/6/94	down	na	305	T08	1	2
Big Beef	adult	11/3/94	up	562	367	T08	2	2
Big Beef	adult	3/18/94	up	558	378	T53	1	2

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Big Beef	adult	3/31/94	down	450	380	T53	2	2
Big Beef	adult	3/18/94	up	440	342	T54	1	2
Big Beef	adult	4/7/94	down	357	338	T54	2	2
Big Beef	adult	3/17/94	up	473	346	T55	1	2
Big Beef	adult	3/30/94	down	371	346	T55	2	2
Big Beef	adult	2/21/95	up	928	430	T55	3	2
Big Beef	adult	2/21/96	up	1388	485	T55	4	2
Big Beef	adult	3/17/94	up	375	346	T56	1	2
Big Beef	adult	3/18/94	down	na	na	T56	2	2
Big Beef	adult	3/16/94	up	383	331	T57	1	2
Big Beef	adult	4/11/94	down	303	330	T57	2	2
Big Beef	adult	3/14/94	up	477	354	T62	1	2
Big Beef	adult	3/29/94	down	439	353	T62	2	2
Big Beef	adult	3/15/95	up	781	419	T62	3	2
Big Beef	adult	4/25/95	down	486	412	T62	4	2
Big Beef	adult	3/14/94	up	489	366	T66	1	2
Big Beef	adult	4/6/94	down	na	362	T66	2	2
Big Beef	adult	1/19/95	up	873	430	T66	3	2
Big Beef	adult	3/30/95	down	680	425	T66	4	2
Big Beef	adult	3/14/94	up	436	347	T68	1	2
Stavis	adult	3/31/94	down	na	345	T68	2	2
Big Beef	adult	3/14/94	up	369	333	T70	1	2
Big Beef	adult	4/19/94	down	301	330	T70	2	2
Big Beef	adult	2/6/95	up	702	400	T70	3	2
Big Beef	adult	4/8/95	down	na	na	T70	4	2
Big Beef	adult	3/11/94	up	381	331	T72	1	2
Big Beef	adult	4/18/94	down	292	328	T72	2	2
Big Beef	adult	4/23/95	down	438	394	T72	3	2
Big Beef	adult	1/11/96	up	867	430	T72	4	2
Big Beef	adult	3/10/94	up	491	353	T76	1	2
Big Beef	adult	1/28/95	up	892	418	T76	2	2
Big Beef	adult	3/10/94	up	551	373	T78	1	2
Big Beef	adult	4/11/94	down	437	370	T78	2	2
Big Beef	adult	3/10/94	up	696	400	T80	1	1
Big Beef	adult	4/19/94	down	588	395	T80	2	1
Big Beef	adult	3/3/94	up	537	364	T95	1	2
Big Beef	adult	4/3/94	down	399	360	T95	2	2
Big Beef	adult	2/28/94	up	526	362	TA0	1	2
Big Beef	adult	4/11/94	down	395	360	TA0	2	2
Big Beef	adult	2/28/94	up	570	403	TA2	1	2
Big Beef	adult	3/1/94	down	565	403	TA2	2	2
Big Beef	adult	2/28/94	up	596	387	TA6	1	1
Big Beef	adult	4/17/94	down	478	379	TA6	2	1
Big Beef	adult	3/21/95	down	919	426	TA6	3	1
Big Beef	adult	2/28/94	up	555	374	TA8	1	2
Big Beef	adult	4/18/94	down	425	368	TA8	2	2
Big Beef	adult	2/6/95	up	795	421	TA8	3	2
Big Beef	adult	4/11/95	down	574	415	TA8	4	2
Big Beef	adult	2/17/96	up	970	452	TA8	5	2
Big Beef	adult	2/28/94	up	645	396	TB0	1	1
Big Beef	adult	4/19/94	down	506	389	TB0	2	1
Big Beef	adult	2/28/94	up	675	412	TB8	1	1

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Big Beef	adult	3/31/94	down	559	412	TB8	2	1
Big Beef	adult	3/1/94	up	486	351	TC9	1	2
Big Beef	adult	1/17/95	up	958	431	TC9	2	2
Big Beef	adult	3/1/94	up	588	388	TD3	1	2
Big Beef	adult	3/4/94	up	na	na	TD3	2	2
Big Beef	adult	4/19/94	down	413	379	TD3	3	2
Big Beef	adult	3/2/94	up	905	451	TD5	1	2
Big Beef	adult	4/8/94	down	656	441	TD5	2	2
Big Beef	adult	3/2/94	up	371	334	TD7	1	1
Big Beef	adult	4/6/94	down	SB	329	TD7	2	1
Big Beef	adult	3/2/94	up	104	220	TE4	1	1
Big Beef	adult	4/6/94	down	SB	212	TE4	2	1
Big Beef	adult	1/29/95	up	290	306	TE4	3	1
Big Beef	adult	11/30/95	up	704	394	TE4	4	1
Big Beef	adult	3/2/94	up	469	359	TE7	1	2
Big Beef	adult	4/3/94	down	317	350	TE7	2	2
Big Beef	adult	2/27/94	up	576	362	TF2	1	1
Big Beef	adult	4/7/94	down	477	359	TF2	2	1
Big Beef	adult	2/27/94	up	596	378	TF4	1	2
Big Beef	adult	4/3/94	down	447	378	TF4	2	2
Big Beef	adult	2/26/94	up	345	331	TF6	1	1
Big Beef	adult	4/11/94	down	291	330	TF6	2	1
Big Beef	adult	2/26/94	up	806	418	TH2	1	2
Big Beef	adult	3/29/94	down	580	411	TH2	2	2
Big Beef	adult	2/26/94	up	529	375	TH6	1	2
Big Beef	adult	4/6/94	down	na	365	TH6	2	2
Big Beef	adult	2/20/96	up	981	459	TH6	3	2
Big Beef	adult	2/26/94	up	441	337	TH8	1	2
Big Beef	adult	4/3/94	down	306	330	TH8	2	2
Big Beef	adult	2/26/94	up	431	347	TJ4	1	1
Big Beef	adult	4/9/94	down	342	344	TJ4	2	1
Big Beef	adult	2/26/94	up	323	320	TJ5	1	1
Big Beef	adult	4/13/94	down	255	314	TJ5	2	1
Big Beef	adult	1/17/95	up	723	401	TJ5	3	1
Big Beef	adult	3/29/95	down	525	395	TJ5	4	1
Big Beef	adult	2/26/94	up	536	365	TJ6	1	2
Big Beef	adult	1/18/95	up	1022	435	TJ6	2	2
Big Beef	adult	2/26/94	up	456	358	TJ8	1	1
Big Beef	adult	3/20/94	down	429	351	TJ8	2	1
Big Beef	adult	2/25/94	up	698	397	TJ9	1	2
Big Beef	adult	4/29/94	down	492	392	TJ9	2	2
Big Beef	adult	1/28/95	up	1012	441	TJ9	3	2
Big Beef	adult	2/25/94	up	355	323	TK1	1	2
Big Beef	adult	4/13/94	down	257	315	TK1	2	2
Big Beef	adult	2/18/94	up	687	400	TK3	1	1
Big Beef	adult	4/7/94	down	496	390	TK3	2	1
Big Beef	adult	2/18/94	up	538	362	TK4	1	2
Stavis	adult	4/19/94	down	na	362	TK4	2	2
Big Beef	adult	1/12/94	up	467	351	TK7	1	2
Big Beef	adult	2/16/94	down	452	352	TK7	2	2
Big Beef	adult	2/28/94	up	395	352	TK7	3	2
Big Beef	adult	2/11/94	up	624	372	TK8	1	1

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Big Beef	adult	2/17/94	up	na	na	TK8	2	1
Big Beef	adult	4/8/94	down	446	363	TK8	3	1
Big Beef	adult	2/6/94	down	662	425	TL6	1	1
Big Beef	adult	4/2/95	down	642	447	TL6	2	2
Big Beef	adult	1/27/94	up	690	401	TL9	1	2
Big Beef	adult	1/31/94	down	580	401	TL9	2	2
Big Beef	smolt	4/11/94	down	62	183	TU1	1	
Big Beef	adult	4/7/95	down	365	356	TU1	2	1
Big Beef	adult	4/10/95	up	360	355	TU1	3	1
Big Beef	adult	4/11/95	down	349	na	TU1	4	1
Big Beef	smolt	4/13/94	down	48	167	TX3	1	
Big Beef	adult	2/1/96	up	474	404	TX3	2	2
Stavis	smolt	6/4/94	down	na	156	Z38	1	
Big Beef	adult	2/5/95	up	523	366	Z38	2	1
Big Beef	adult	1/15/96	up	990	443	Z38	3	1
Big Beef	adult	11/9/94	up	335	321	Z49	1	1
Big Beef	adult	4/22/95	down	267	321	Z49	2	2
Big Beef	adult	11/14/94	up	629	392	Z52	1	1
Big Beef	adult	2/26/95	up	568	394	Z52	2	1
Big Beef	adult	1/17/95	up	484	352	Z61	1	2
Big Beef	adult	5/2/95	down	332	345	Z61	2	2
Big Beef	adult	1/18/95	up	440	335	Z62	1	2
Big Beef	adult	3/28/95	down	335	333	Z62	2	2
Big Beef	adult	1/15/96	up	822	417	Z62	3	2
Big Beef	adult	3/14/96	down	567	398	Z62	4	2
Big Beef	adult	1/18/95	up	524	369	Z63	1	1
Big Beef	adult	4/4/95	down	382	364	Z63	2	1
Big Beef	adult	1/29/95	up	838	414	Z64	1	2
Big Beef	adult	3/20/95	down	631	409	Z64	2	2
Big Beef	adult	2/2/95	up	na	356	Z70	1	1
Big Beef	adult	3/22/95	down	341	348	Z70	2	1
Big Beef	adult	2/3/95	up	203	269	Z73	1	1
Big Beef	adult	4/5/95	down	163	264	Z73	2	1
Big Beef	adult	2/3/95	up	425	337	Z76	1	2
Big Beef	adult	5/1/95	down	270	332	Z76	2	2
Big Beef	adult	2/5/95	up	518	361	Z79	1	2
Big Beef	adult	4/1/95	down	388	352	Z79	2	2
Big Beef	adult	2/5/95	up	300	317	Z82	1	2
Big Beef	adult	3/29/95	down	226	313	Z82	2	2
Big Beef	adult	11/20/95	up	648	396	Z82	3	2
Big Beef	adult	2/5/95	up	835	419	Z87	1	2
Big Beef	adult	4/7/95	down	654	415	Z87	2	2
Big Beef	adult	2/6/95	up	743	409	Z88	1	2
Big Beef	adult	1/3/96	up	946	434	Z88	2	2
Big Beef	adult	2/6/95	up	413	328	Z89	1	2
Big Beef	adult	4/8/95	down	na	na	Z89	2	2
Big Beef	adult	2/6/95	up	997	443	Z90	1	2
Big Beef	adult	3/18/96	down	743	458	Z90	2	2
Big Beef	adult	2/6/95	up	544	363	Z91	1	2
Big Beef	adult	3/22/95	down	406	365	Z91	2	2
Big Beef	adult	2/6/95	up	657	387	Z92	1	2
Big Beef	adult	3/29/95	down	495	383	Z92	2	2

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Big Beef	adult	2/8/95	up	362	311	Z94	1	2
Big Beef	adult	1/7/96	up	626	380	Z94	2	2
Big Beef	adult	4/12/96	down	446	378	Z94	3	2

*Indicates the order and count of capture events for each individual.

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Refereed journal articles

Wenburg JK, George GW (1995) Placement of visible implant tags in the anal fin of wild coastal cutthroat trout. North American Journal of Fisheries Management, 15, 874-877.

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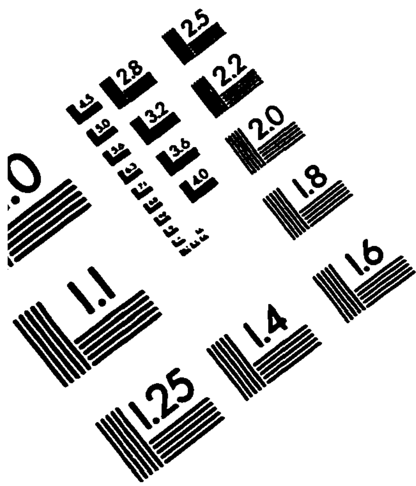
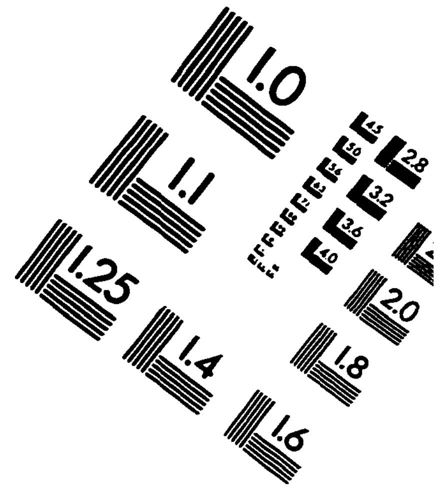
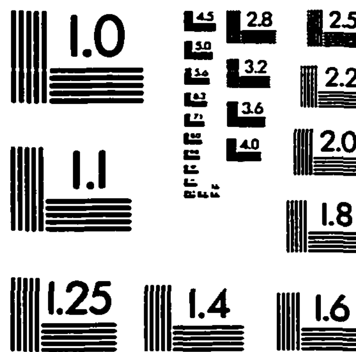
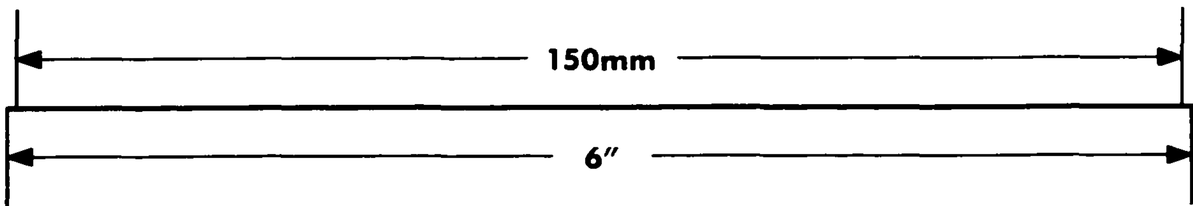
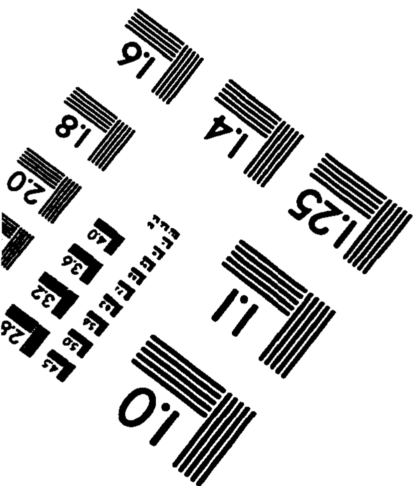
IMAGE EVALUATION TEST TARGET (QA-3)











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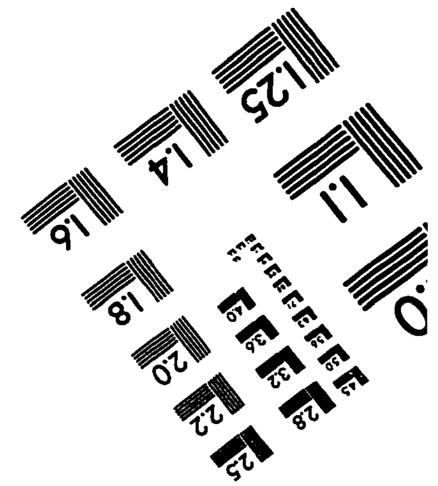

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