Exxon Valdez Oil Spill
Restoration Project Final Report

Relations Between Dolly Varden Populations and Between Coastal Cutthroat Trout Populations in Prince William Sound

Restoration Project 98145
Final Report

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Study History: Project 98145 began in FY96 as a Monitoring and Research study. Subsequent years of study were funded under projects 97145, 98145, and 99145. The purpose of the study was to determine the genetic structure of Dolly Varden and coastal cutthroat trout populations in Prince William Sound, which could be used to develop species-specific recovery programs. Samples were collected from selected populations of Dolly Varden and coastal cutthroat trout from sites in Prince William Sound in FY96 and FY97. Genetic and meristic analyses were conducted from FY97 to FY99. Annual reports were submitted in FY97, FY98, and FY99. The final report was submitted in FY2000. This report is a revision of the final report based on new analysis of the data.

Abstract: We examined genetic, meristic, and otolith microchemistry variation in coastal cutthroat trout and Dolly Varden charr in Prince William Sound. Geographically proximate aggregations of coastal cutthroat trout around Orca Bay were genetically similar and had moderate rates of genetic exchange. Genetic divergence increased in geographically more distant populations from Columbia Bay, Unakwik Inlet, Gunboat Lakes, and Green Island, which had reduced levels of genetic exchange and diversity. This pattern was best explained by isolation by distance. Dolly Varden found below barriers were characterized by high genetic exchange and minor genetic differences with no strong geographical patterns of differentiation, but populations above barriers were genetically different. Otolith microchemistry did not show differences in Sr/Ca ratios of otolith primordial between above- and below-barrier groups but did show patterns consistent with resident or anadromous life histories. Inferences about population histories, abundances, and patterns of dispersal from these data were the basis of recommendations for recovery programs for these species.

Key Words: Coastal cutthroat trout, Dolly Varden, *Exxon Valdez* oil spill, meristics, *Oncorhynchus clarki*, population genetics, Prince William Sound, recovery plan, *Salvelinus malma.*

Project Data: *Description of data* — Genetic data are allozymes, mitochondrial DNA, and microsatellite DNA frequencies from each species. Meristic data are counts of selected anatomical features of each species. Otolith data are strontium/calcium ratios. *Format* — Allele or haplotype frequencies; meristic counts; Sr/Ca ratios. *Custodian* — Contact Kitty Griswold (USGS/BRD, S.O. Conte Anadromous Fish Research Center, One Migratory Way, P.O. Box 796, Turner Falls, MA 01376, phone: 413-863-3821, fax: 413-863-9810, e-mail: kitty_griswold@usgs.gov, web address: www.lsc.usgs.gov). *Availability* — Data are available on diskette and will be available in peer-reviewed publications.

Citation: Currens, K. P., K. E. Griswold, and G. H. Reeves. 2003. Relations between Dolly Varden populations and between coastal cutthroat trout populations in Prince William Sound, *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 98145), USDA Forest Service, Pacific Northwest Research Station, Corvallis, Oregon.

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Executive Summary

Genetic and ecological differences among populations and species can influence the success of conservation and recovery strategies. In Prince William Sound (PWS), both Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*) were injured by the *Exxon Valdez* oil spill and are a focus of conservation and recovery planning. Similarities between the species led to similar kinds of impacts. Both species breed in coastal streams and rivers, migrate to salt water to rear where they feed on amphipods and benthic epifauna injured by the oil spill, and return to freshwater habitats to overwinter or breed. Resident forms may spend their entire life in freshwater, often above barriers such as waterfalls that limit the upstream migration of anadromous fish. Similar life histories and habitat occupied by these fishes may also suggest that the same conservation and recovery strategy should be appropriate for these species. In contrast, differences in capacity to adapt to environmental changes, to disperse, and to colonize habitat, which affect how well the species recover from natural or human caused disturbances, may require different approaches for the two species.

Dolly Varden and coastal cutthroat trout in PWS have had to colonize, survive, and adapt to a highly dynamic landscape. Although they successfully colonized the same environment, coastal cutthroat trout and Dolly Varden have important differences that may affect their persistence in PWS. Zoogeographically, PWS is the northern edge of the distributional range of coastal cutthroat trout. In contrast, it is the center of the range for Dolly Varden. Distribution of coastal cutthroat trout within PWS tends to be patchy, whereas Dolly Varden are ubiquitous. In PWS, Dolly Varden may disperse extensively during their marine migration. In contrast, dispersal of coastal cutthroat trout may be more restricted.

To help identify recovery strategies for coastal cutthroat trout and Dolly Varden in PWS, we examined the genetic structure of each species for different patterns of isolation by distance, isolation above barriers, and parapatric differentiation associated with ecological differences in natal streams. Specifically, we had three objectives:

(1) Determine for both Dolly Varden and coastal cutthroat trout if populations in PWS were a single population or are separate subpopulations;
(2) Determine for both Dolly Varden and coastal cutthroat trout whether resident and amphidromous migratory forms in a watershed were a single population or separate populations; and
(3) Develop a restoration strategy for each species based on results from objectives 1 and 2.

To test for genetic population structure, we examined variation at allozyme, mitochondrial DNA (mtDNA), and microsatellite DNA loci and subsequently focused one or two of these of these for data for most of the study. We also investigated meristic variation in classical taxonomic traits for correspondence with geographical patterns of genetic variation. To look for evidence of generational changes in resident or amphidromous migratory strategies, we examined microchemical patterns in otoliths.

All of our analyses pointed to the same conclusion about geographical genetic structure of coastal cutthroat trout in Prince William Sound. Geographically proximate aggregations around Orca Bay (Milton Lake and locations on Hawkins and Hinchinbrook islands) were genetically most similar and had high rates of genetic exchange, but genetic divergence increased in

geographically more distant populations around Prince William Sound. The most divergent aggregations were those from northwestern PWS in Columbia Bay, Unakwik Inlet, Gunboat Lakes, and Green Island, which had the lowest levels of genetic exchange and genetic diversity.

We found no evidence that genetic divergence in coastal cutthroat trout was due to secondary contact after historical vicariance, allopatric divergence of populations above and below barriers, or parapatric divergence associated with ecological differences in natal freshwater habitats. Lack of genetic differences between above and below barrier populations was supported by otolith microchemistry, which showed no difference in the mean Sr/Ca ratios in the primordial region of otoliths. In contrast, we found a strong pattern of isolation by distance, following a one-dimensional stepping stone model for dispersal along shorelines of Prince William Sound. Overall, our results suggested that gene flow and genetic drift were approximately at equilibrium in PWS coastal cutthroat trout, but that recent colonizations in Columbia Bay, Unakwik Inlet, and perhaps other aggregations may be resetting long-term patterns of isolation by distance.

Despite similarities in their life history, Dolly Varden in PWS had different patterns of genetic variation than coastal cutthroat trout. Isolation and allopatric divergence of above-barrier populations was the most important source of genetic differences in Dolly Varden. Overall, differentiation was nearly twice as large for above- and below-barrier populations ($\theta_S = 0.101$) than for only below-barrier aggregations in PWS ($\theta_S = 0.040$) and above-barrier populations in Power and Hawkins creeks had fewer microsatellite alleles per locus and lower heterozygosities than those below. We found no significant differences in Sr/Ca ratios in the primordial region between the two groups. Below-barrier Dolly Varden, however, had higher Sr/Ca ratios in the outer margin of the otolith, which is consistent with deposition during its marine phase, whereas Sr/Ca ratios of above-barrier individuals remained low, which is consistent with a prolonged freshwater residency.

Below barriers Dolly Varden were characterized by large amounts of genetic exchange and minor genetic differentiation. We saw no strong geographical patterns of differentiation. We found no evidence that genetic divergence in Dolly Varden was due to secondary contact after historical vicariance or parapatric divergence associated with ecological differences in natal freshwater habitats in allozyme or microsatellite DNA variation, but we did find mitochondrial DNA differences between Dolly Varden from different habitats. These may indicate sexual dimorphism in dispersal behavior or potential sampling errors. We found weak evidence of isolation by distance in Dolly Varden. Patterns of isolation by distance among below-barrier aggregations occurred at shorter distances (< 100 km) with large levels of genetic exchange that prevented extensive population differentiation.

The similar life histories of Dolly Varden and coastal cutthroat trout in PWS indicated that recovery programs for populations that were injured in the spill should have similar components. The emphasis on the components for each of the species should vary, however, to reflect the differences in distribution, migration, genetic structure, and population sizes between the two species. Five aspects of recovery programs are (1) status assessments, (2) habitat protection, (3) habitat restoration, (4) harvest regulations, and (5) population reintroductions or artificial propagation.

The status of each species should be determined before restoration programs are undertaken and at regular periods during the recovery. A key element is compiling a thorough assessment of the current distribution of each species and the available habitat, including populations above barriers. With such information, it may be possible to use the relationships between independent populations, migration, and geographical distance from our analyses to quantitatively assess potential for natural recovery, colonization, or extinction of injured populations. This information would also allow comparisons of injured populations with non-injured populations that are in the same general area, as recommended by the EVOS Trustee Council.

Protection of freshwater habitat is more efficient than recovery of damaged habitat. Based on our results, priorities for habitat protection for coastal cutthroat trout should focus (1) on watersheds with properly functioning cutthroat trout habitat and healthy populations adjacent (within 30 km shoreline distance) to injured populations, (2) well-developed cutthroat habitat with injured populations or unoccupied habitat adjacent to healthy populations, and (3) other healthy populations. For Dolly Varden, watersheds with injured populations should be the priorities for protection, as long as abundance and distribution of healthy populations are not simultaneously reduced. Populations above barriers are a second important priority.

Restoration activities should be directed towards increasing habitat complexity, which will help maintain equilibrium between gene flow and genetic drift in coastal cutthroat trout and may encourage subpopulation differentiation in Dolly Varden. Complexity can be increased immediately by the addition of large wood. At the same time, riparian zones should be restored to provide future sources of wood.

Harvest has a direct impact on population abundance, population growth, and dispersal. We recommend that harvest of coastal cutthroat trout in PWS should be restricted in all areas and possibly eliminated in areas with injured populations. In contrast, regulation of harvest for Dolly Varden does not need to be as widespread or extensive, because the high migration rate among aggregations and larger population sizes should help buffer effects of harvest.

Given the above strategies, rebuilding injured populations through transfers from other streams or artificial propagation should not be necessary to recover injured populations of either species. We expect, however, that recovery of injured coastal cutthroat trout populations will be slow relative to Dolly Varden. Cutthroat trout populations were characterized by greater geographical isolation, genetic divergence, and small population sizes. These factors suggest that recovery of injured populations depends less on nearby populations than on the productivity of the injured population and the extent that it was injured by the oil spill. In contrast, injured Dolly Varden population should recover more quickly than injured coastal cutthroat trout populations, because higher rates of immigration should contribute to the recovery process. The primary factor determining the rate of recovery of damaged populations of both species will likely be the recovery of the environment.

Introduction

Genetic and ecological differences among populations and species can influence the success of conservation and recovery strategies. In Prince William Sound (PWS), both Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*) were injured by the *Exxon Valdez* oil spill and are a focus of conservation and recovery planning. Similarities between the species led to similar kinds of impacts. Dolly Varden and coastal cutthroat trout in oiled areas, for example, had lower survivals in 1989-1990 and lower growth rates in 1989-1990 and 1990-1991, respectively (Hepler et al. 1993), in part because of chronic starvation related to impacts on similar prey. In nearshore areas, both species feed on amphipods and the benthic epifauna, which are especially susceptible to petrogenic hydrocarbons (Teal and Howarth 1984) and which decreased in areas exposed to the spill (Jewett and Dean 1993, Jewett et al. 1993). Similar life histories and habitat occupied by these fishes may also suggest that the same conservation and recovery strategy should be appropriate for these species. In contrast, differences in capacity to adapt to environmental changes, to disperse, and to colonize habitat, which affect how well the species recover from natural or human caused disturbances, may require different approaches for the two species. Patterns of genetic differences among populations provide clues to whether these differences exist and their magnitudes (Allendorf et al. 1987). Consequently, identifying genetic differences and understanding their patterns is important for designing successful conservation and recovery strategies.

Dolly Varden and coastal cutthroat trout, which are found throughout PWS, Alaska (Mills 1988), share similar life history characteristics. They occupy much of the same habitat, which consists of gravelly, low coastal streams and rivers, lakes, and estuaries and nearshore marine areas (Scott and Crossman 1979). Unlike their anadromous Pacific salmon cousins, which migrate to salt water to rear and return to freshwater habitats to breed, both Dolly Varden and coastal cutthroat trout display more primitive amphidromous behavior (Stearley 1992): they breed in freshwater and may migrate to salt water, but they will return to freshwater for reasons other than breeding. Both species also have migratory, marine life history forms and resident, freshwater life history forms. After hatching in freshwater, Dolly Varden and cutthroat trout may spend up to four years in streams or lakes before migrating to the marine environment where they feed in nearshore and estuary areas during the spring and summer (Scott and Crossman 1979, Morrow 1980). Both species feed on amphipods, benthic epifauna, and other fish (Narver and Dahlberg 1965, Armstrong 1971). Unlike Pacific salmon, which remain in salt water until they return to their natal streams to spawn, amphidromous Dolly Varden and coastal cutthroat trout may enter non-natal streams during the winter months before returning to the estuary in the spring (Bernard et al. 1995, Jones and Seifert 1997). Resident forms, in contrast, spend their entire life in freshwater. They often occur above barriers, such as waterfalls, that limit the upstream migration of anadromous fish, although downstream migration may still occur.

Dolly Varden and coastal cutthroat trout in PWS have had to colonize, survive, and adapt to a highly dynamic landscape. Prince William Sound is a large estuary occupying nearly 39,000 km^2 and 2800 km of shoreline (Figures 1, 2). It is surrounded by the Kenai and Chugach mountain ranges and contains an extensive subarctic archipelago that protects it from the high-energy Gulf of Alaska, deep fjords, and over 150 active glaciers (including 20 tidewater glaciers) that continue to sculpt much of the landscape and affect steams. In addition, it sits over the subduction zone of the Pacific Plate (von Huene 1989), which creates frequent earthquakes,

landslides, and mudflows that repeatedly reshape streams and fish habitat. Prince William Sound was the epicenter of the largest earthquake ever recorded (9.2 Richter scale). In four minutes, the earthquake lowered streams west of the fault by 2.3 m and raised areas east (Montague Island) as much as 10 m, generated tsunamis up to 10 m high, and caused large mudslides (Plafker 1969, 1990). Extreme earthquakes have probably occurred here every 1000 years, but smaller ones are much more frequent (Plafker et al. 1992)

Both Dolly Varden and coastal cutthroat trout colonized this region from other areas as Pleistocene glaciers receded and streams became suitable for breeding and rearing. The timing of this colonization is unknown, but glaciers have continued to affect rates of extinction and recolonization up to present times. During the last glacial maximum (25,000-10,000 BP), glaciers covered Prince William Sound out to the continental shelf (Mann and Peteet 1994). The ice began to recede about 14,000 BP (Sirkin and Tuthill 1987, Mann and Hamilton 1995), exposing the outer islands, but it persisted in some areas until 10,000 BP (Ager 1992, 1999). Sedges, willows, and ferns began colonizing inner fjords of PWS by 10,000 BP (Heusser 1983, 1985). Between 10,000-6,000 BP, the climate was warmer and drier than today and glaciers probably retracted to nearly present day positions (Calkin 1988, Wiles and Calkin 1990). During the late Holocene, however, glaciers advanced three times: first about 3700 BP; later from 500-900 AD; and most recently from 1200-1890 AD (Wiles and Calkin 1990, 1993, 1994; Calkin et al. 2001). Although periods of retreating land glaciers may have provided opportunity for colonization in some areas, the numerous tidewater glaciers on the mainland may have limited dispersal. Tidewater glaciers surge and retreat asynchronously with land glaciers because they respond to nonclimatic variables (Mann 1986, Meier and Post 1987).

The eastern and outer islands of PWS may have provided the earliest opportunities for colonization. These areas were free of ice first and fish habitat here would have been less affected by turbid outflows of retreating land glaciers and surging of tidewater glaciers. Salmonids do not colonize cold, turbid glacial streams (Milner and Bailey 1989) and rapid down-cutting in recently deglaciated streams hinders persistence of large numbers of fish (Benda et al. 1992). As sediment loads in streams decline and large woody debris from forests accumulates, colonization is more likely (Benda et al. 1992). In recent colonizations, Dolly Varden were among the first fishes to colonize main stems of streams following deglaciation (Milner et al. 2000, Milner and York 2001). In PWS, coastal forest vegetation that helped create fish habitat generally spread westward across PWS to the eastern Kenai (Ager 1999). Sitka spruce (*Populus trichocarpa*) and mountain hemlock (*Picea sitchensis*), which characterize these forests, did not arrived until 3000 BP in PWS and did not assume a major role in inner fjords until 2000 BP, although they may have been established on outer islands earlier (Heusser 1983, Ager 1999).

Although they successfully colonized the same environment, coastal cutthroat trout and Dolly Varden have important differences that may affect their persistence in PWS. Zoogeographically, PWS is the northern edge of the distributional range of coastal cutthroat trout. In contrast, it is the center of the range for Dolly Varden (Scott and Crossman 1979). Distribution of coastal cutthroat trout within PWS tends to be patchy, whereas Dolly Varden are ubiquitous. In PWS, Dolly Varden may disperse extensively during their marine migration (McCarron and Hoffman 1993, Bernard et al. 1995). In contrast, dispersal of coastal cutthroat trout may be more restricted (McCarron and Hoffman 1993).

Metapopulation theory predicts that at the edge of a species range, population persistence fluctuates between extinctions and establishment of new populations (Hoffman and Blows 1994). Although coastal cutthroat trout in PWS are currently at the edge of the species range, PWS would also have been at the edge of the range for Dolly Varden when it became ice free and Dolly Varden were colonizing from Pleistocene glacial refugia. After a species expands, isolation by distance—where dispersal distance between populations limits the exchange of individuals among populations—may eventually lead to a progressive pattern where the amount of genetic differentiation is related to geographical distance (Wright 1943, Kimura and Weiss 1964, Slatkin 1991, 1993, Rousset 1997). The pattern of isolation by distance and levels of genetic diversity provide clues to the dynamics of these populations. Where populations have recently colonized an area and establishment of new populations exceeds extinctions, lack of equilibrium between recent, large levels of gene flow (estimated as the number of reproducing migrants exchanged among populations) and genetic drift means that patterns of isolation by distance will occur for only nearby locations (Slatkin 1993) and genetic diversity may be reduced in newly founded populations (Nagylaki 1976). As populations reach equilibrium, the number of migrants per generation ($\hat{M}$) decreases with increasing geographical distance (d). The slope of the regression of $\log_{10}(\hat{M})$ against $\log_{10}(d)$ may also indicate the spatial dimension of dispersal. If dispersal follows a simple one-dimensional stepping stone model, such as between adjacent populations in a circle, the slope is expected to be approximately –1.0; under a more complex two-dimensional stepping stone model, it will be approximately –0.5 (Slatkin and Maddison 1990, Slatkin 1991). Over time, rate of extinctions may exceed establishments and ranges may contract. Increasing fragmentation leads to patchy, small, peripheral populations with low levels $\hat{M}$ but no pattern of isolation by distance (Slatkin 1993). Bottlenecks in abundance may also lead to loss of genetic diversity.

Patterns of genetic diversity in Dolly Varden and coastal cutthroat trout may also reflect two additional phenomena: (1) secondary contact after historical vicariance and (2) parapatric divergence associated with ecological differences in natal freshwater environments. Salmonids that colonized the glaciated coastline of the Pacific Northwest generally came from a refuge to the north in Beringia or from southern Columbia (Pacific) refuges (McPhail and Lindsey 1970, 1986; Taylor et al. 1999, Redenbach and Taylor 2002). Redenbach and Taylor (2002) explained genetic patterns in Dolly Varden in deglaciated areas of British Columbia as a secondary contact between two clades of Dolly Varden associated with northern and southern refugia. Likewise, Wenburg et al. (1998) speculated that a pattern of high heterozygosity but low gene flow in coastal cutthroat trout in Washington State was the result of secondary contact of isolated remnants of the subspecies after the retreat of the Puget Sound glacial lobe.

On a smaller geographical and temporal scale of vicariance are the complex dynamics between resident coastal cutthroat trout and Dolly Varden isolated above and below barriers. Barriers may disrupt populations during episodes of tectonic or glacial activity. Upstream barriers to gene flow may lead to genetic differentiation, but resident populations may also continue to contribute to the amphidromous populations, either through downstream migration or episodically as barriers fail. Patterns of differentiation and contribution vary. Studies of sympatric populations of closely related resident and anadromous life history forms of rainbow trout (*O. mykiss*) suggest that one form may give rise to the other or not. Sympatric forms of resident and anadromous forms of

rainbow trout were reproductively isolated in the Deschutes River, Oregon (Currens et al. 1990, Zimmerman and Reeves 2000), but in the Babine River, British Columbia, some anadromous individuals were of resident origin and visa versa (Zimmerman and Reeves 2000). No consistent pattern of genetic differentation was present between isolated and unisolated populations of coastal cutthroat trout in a southern Oregon river system (Griswold 1996) and rainbow trout across much of its range (Currens 1997).

Genetic structure may also reflect parapatric differentiation associated with ecological differences in natal freshwater environments. Parapatric differentiation occurs when populations that share portions of a geographical area with limited interbreeding evolve differences. The tendency of salmonids to return to the natal streams to spawn (Scheer 1939, Ricker 1972, Quinn 1993) limits gene flow without physical barriers and allows for adaptations to different local environments (Taylor 1991). In the Kenai and Kasilof rivers, Alaska, for example, mitochondrial DNA and allozyme differences among parapatric populations of chinook salmon (*Oncorhynchus tshawytscha*) were associated with ecological differences in temperature and stream size (tributary versus mainstem) of spawning habitat (Adams et al. 1994). Homing tends to be more precise in salmonids with longer freshwater residence and limited saltwater migrations (reviewed in Quinn 1993). Consequently, limited marine migrations of Dolly Varden and coastal cutthroat trout suggest that they may home more precisely and limited gene flow would allow for genetic structure to arise that is associated with environmental differences among natal streams. Although most studies of Dolly Varden have focused on their taxonomic relationships to the closely related bull trout (*Salvelinus confluentus)* or their post-Pleistocene distribution and differentiation (e.g., Taylor et al. 2001, Redenbach and Taylor 2002). Krueger et al. (1999) found that 8% of the allozyme variation in Dolly Varden in the Beaufort Sea could be attributed to differences among local populations from different watersheds and they suggested that precise homing might occur to different watersheds. The authors also noted, however, that mixtures of Dolly Varden populations occurred at moderately long distances (350 km), which is consistent with their long distance dispersal capacity detected through tagging studies (DeCicco 1992). Likewise, genetic investigations of coastal cutthroat trout have shown differentiation among regional groups (Johnson et al. 1999), among populations from different watersheds (Campton and Utter 1987, Wenberg et al. 1998, Wenberg and Bentzen 2001), and among some populations within a watershed isolated by barriers to migration (Griswold et al. 1997).

Objectives

To help identify recovery strategies for coastal cutthroat trout and Dolly Varden in PWS, we examined the genetic structure of each species for different patterns of isolation by distance, isolation above barriers, and parapatric differentiation associated with ecological differences in natal streams. Specifically, we had three objectives:

(1) Determine for both Dolly Varden and coastal cutthroat trout if populations in PWS were a single population or are separate subpopulations;
(2) Determine for both Dolly Varden and coastal cutthroat trout whether resident and amphidromous migratory forms in a watershed were a single population or separate populations; and
(3) Develop a restoration strategy for each species based on results from objectives (1) and (2).

Methods

To better understand the genetic and life history factors that might affect recovery strategies for coastal cutthroat trout and Dolly Varden, we used five kinds of data. To test for genetic population structure, we examined variation at allozyme, mitochondrial DNA (mtDNA), and microsatellite DNA loci and subsequently focused on one or two of these of these for data for most of the study. We also investigated phenotypic patterns of meristic variation in classical taxonomic traits for correspondence with geographical patterns of genetic variation. To look for evidence of generational changes in resident or amphidromous migratory strategies, we examined microchemical patterns in otoliths.

The three kinds of genetic data have different practical and theoretical advantages and disadvantages. The main advantages of examining allozyme variation are that the techniques and interpretation have been well-developed for salmonids (Aebersold et al. 1987) and thirty years of extensive research has shown that allozyme variation can detect genetic population structure (e.g., Allendorf 1975, Kristiansson and McIntyre 1976, Berg and Gall 1988, Utter et al. 1989, Reisenbichler et al. 1992, Weitkamp et al. 1995, Currens 1997). Allozyme loci are moderately variable in salmonids (usually 1-5 alleles per locus). Consequently, it is essential to use many loci to adequately describe population structure. A disadvantage is that allozyme techniques usually require lethal sampling to collect different tissues (muscle, heart, liver, and eye) needed to examine a broad suite of loci (>30).

In contrast, both mtDNA and microsatellite DNA do not require lethal sampling. The main advantage of mitochondrial DNA variation is that it allows investigators to examine both the geographical distribution of mtDNA haplotypes in populations and the evolution of the DNA molecule to resolve broad phylogenetic and biogeographical patterns of evolution (Avise 1994). In our review of the published literature on mtDNA variation in salmonids, for example, we found that 80% of the publications used phylogenetic differences among major clades to resolve questions focused on systematics (e.g., Berg and Ferris 1984, Shedlock et al. 1991, Shed'ko et al. 1996), specific and subspecific hybridization (e.g., Gyllensten et al. 1985, Wilson and Bernatchez 1998, Taylor et al. 2001), vicariance and post-Pleistocene colonization (e.g., Nielsen et al. 1994b, Danzmann et al. 1998, Wilson and Hebert 1998, McCusker et al. 2000, Bernatchez 2001), and origins and effects of hatchery introductions (e.g., Nielsen et al. 1994a, Williams et al. 1997, Hansen et al. 2000). Mitochondrial DNA is moderately variable in salmonids. Although mtDNA may evolve more rapidly than many nuclear genes (Brown et al. 1982), it is haploid and clonally inherited through maternal lines (Gyllensten et al. 1991), which makes the genetic effective size for mtDNA one-fourth that of nuclear genes. Consequently, rare haplotypes are vulnerable to loss through genetic drift. Because mtDNA is maternally inherited, a significant disadvantage of mtDNA is that inferences about dispersal and gene flow based on only mtDNA variation may not be appropriate if males and females do not have the same migratory behavior.

Unlike allozyme and mtDNA variation, microsatellite DNA may be highly variable in salmonids. The rapid mutation rate and greater number alleles per locus for microsatellite DNA variation compared to allozyme or mtDNA variation make microsatellite DNA more useful for studying closely related populations, family structure, and effects of recent population bottlenecks (Takezaki and Nei 1996).

Meristic variation was used by taxonomists to study intraspecific population structure before the advent of biochemical and molecular techniques (e.g, Behnke 1992). Meristic variation reflects both genetic and environmental control (Leary et al. 1985) and may show congruent patterns with molecular analyses (e.g.,Currens 1997, Currens et al. 1997). When geographical patterns in meristic variation are congruent with molecular patterns, they provide supporting evidence of population structure.

Unlike the allozyme, mtDNA, microsatellite DNA, and meristic data, otolith microchemistry does not reflect genetic variation. Differences in chemical composition of maternal environments, however, may be reflected in the microchemistry of otoliths in offspring and potentially used to examine a fish's age, growth, and whether the maternal parent was resident or amphidromous (Radtke 1989, Gunn et al 1992). Otoliths are formed by successive growth of concentric rings of calcium carbonate and trace elements from the environment around dense primordia in otoliths beginning with development of eggs. Strontium (Sr) is freely substituted for calcium (Ca) during deposition in otoliths in proportion to its concentration in the environment. Because marine environments have elevated levels of Sr relative to most freshwater environments, larger Sr/Ca ratios in otoliths are a signature of movement of an individual from freshwater to saltwater (Kalish 1990).

Sample Collection

We collected coastal cutthroat trout from 13 locations, including two that were above presumed barriers to upstream migration (Figure 1, Table 1) and Dolly Varden from 16 locations, including three above presumed barriers (Figure 2, Table 2) in 1996 and 1997 from PWS and the Copper River Delta. Because little was known about the distribution and abundance of coastal cutthroat trout and Dolly Varden in PWS, we selected potential sampling sites by consulting with fish biologists from the Alaska Department of Fish and Game (ADFG), the U.S. Forest Service (USFS), and other local experts to identify aggregations that would be large enough to be sampled without having potential negative consequences. Sample sizes were limited by agreement with ADFG to reduce risk to the populations but were similar to those for other recent genetic studies on potentially vulnerable species (Kanda and Allendorf 2001, Wenberg and Bentzen 2001). At two sites where coastal cutthroat trout were sampled, Copper River and Makaka Creek (Figure 1, Table 1), we only collected non-lethal fin clips for DNA analyses because of the potentially vulnerable status of the populations. Subsequent preliminary analyses of allozyme variation from seven locations sampled in July of 1996 suggested that relative low levels of variation in PWS coastal cutthroat trout compared to other areas (Reeves et al. 1996) might not provide adequate power to detect population structure. Consequently, this sampling effort was not expanded to more sites and additional years.

Coastal cutthroat trout were collected primarily in July and Dolly Varden in September with baited minnow traps, seines, electroshockers, and hook and line. Collections below barriers were made near the estuary as each species returned to freshwater to overwinter or spawn. Fish were anesthetized with a lethal dose of MS-222 and tagged with a unique identification number. For fish >250 mm in length, muscle, eye, liver, and heart tissue were removed immediately, placed in

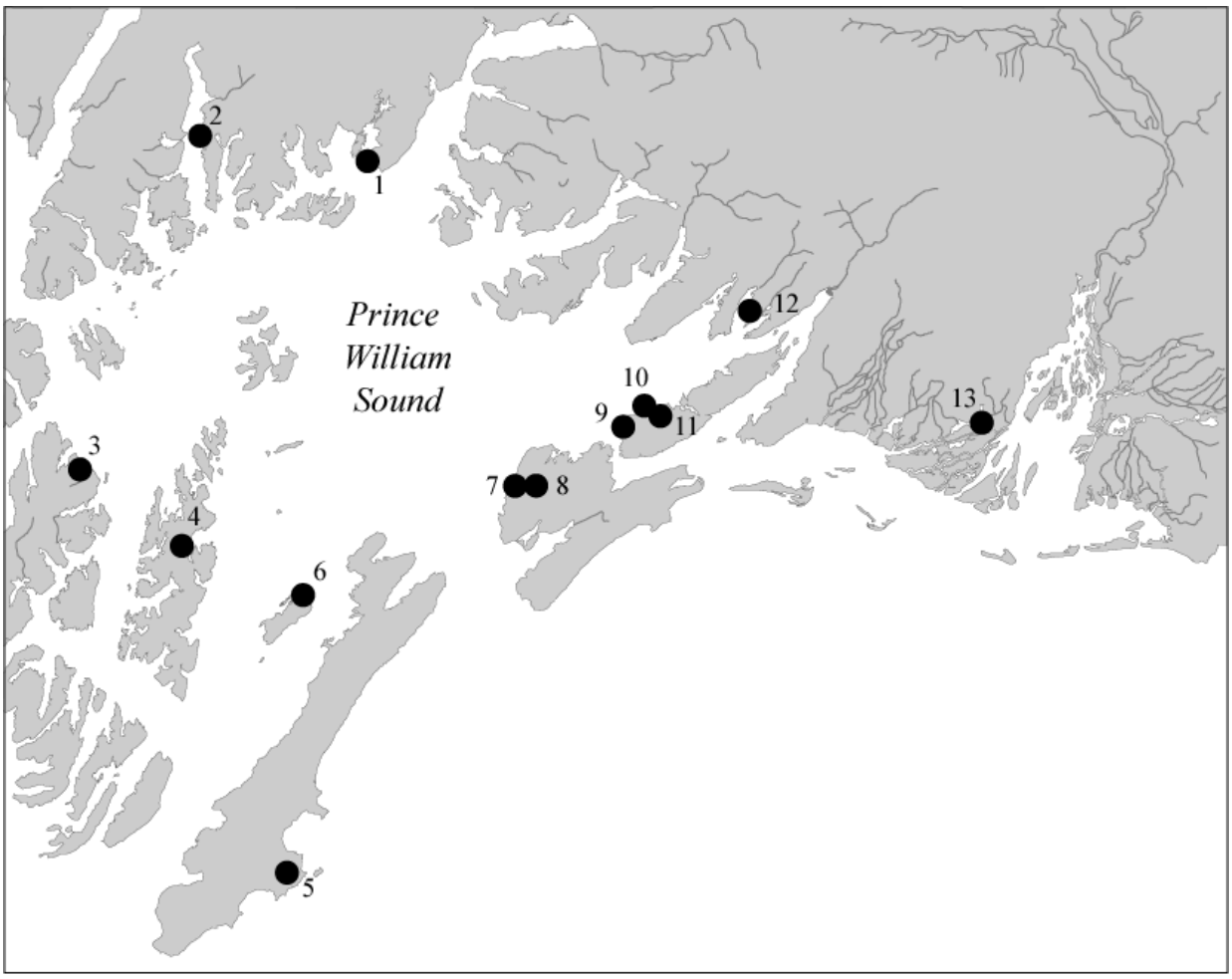


Figure 1. Sampling locations of coastal cutthroat trout collected in 1996 and 1997 in Prince William Sound, Alaska. Codes are (1) Columbia Bay; (2) Unakwik Inlet; (3) Gunboat Lakes; (4) West Arm, Bay of Isles; (5) Stump Lake; (6) Green Island; (7) Shelter Bay-below barrier; (8) Shelter Bay-above barrier; (9) Makaka Creek; (10) Hawkins Creek-below barrier; (11) Hawkins Creek-above barrier; (12) Milton Lake; and (13) Copper River, Eighteen Mile Creek.

Table 1. Location, sample size, latitude and longitude, and natal habitat classification of 1996 and 1997 collections of coastal cutthroat trout in Prince William Sound, Alaska. Natal habitats are classifications used for tests of population structure associated ecological differences. Island habitat refers to locations in the Prince William Sound Islands ecosection (see text for details). Site number refers to location in Figure 1. ** denotes sites used for mtDNA analysis.

	Sample Size		Location			
Name (site number)	(1996)	(1997)	Lat.	Lon.	Natal Habitats	
Columbia Bay (1)	0	20	60.954	147.006	East	Mainland
** Unakwik Inlet (2)	20	20	61.022	147.518	West	Mainland
** Gunboat Lakes (3)	20	20	60.500	147.972	West	Mainland
Knight Island						
West Arm, Bay of Isles (4)	0	20	60.391	147.717	West	Island
Montague Island						
** Stump Lake (5)	20	20	59.873	147.472	West	Island
Green Island						
Green Island Creek (6)	4	13	60.284	147.410	West	Island
Hinchinbrook Island						
** Shelter Bay-below barrier (7)	20	20	60.428	146.659	East	Island
** Shelter Bay-above barrier (8)	30	27	60.414	146.580	East	Island
Hawkins Island						
Makaka Creek (fin clips) (9)	20	20	60.505	146.292	East	Island
** Hawkins Creek, below barrier (10)	20	20	60.521	146.196	East	Island
** Hawkins Creek, above barrier (11)	25	12	60.501	146.113	East	Island
** Milton Lake (12)	20	23	60.623	145.878	East	Mainland
Eighteen Mile Creek, (fin clips) (13)	20	0	60.496	145.072	East	Mainland

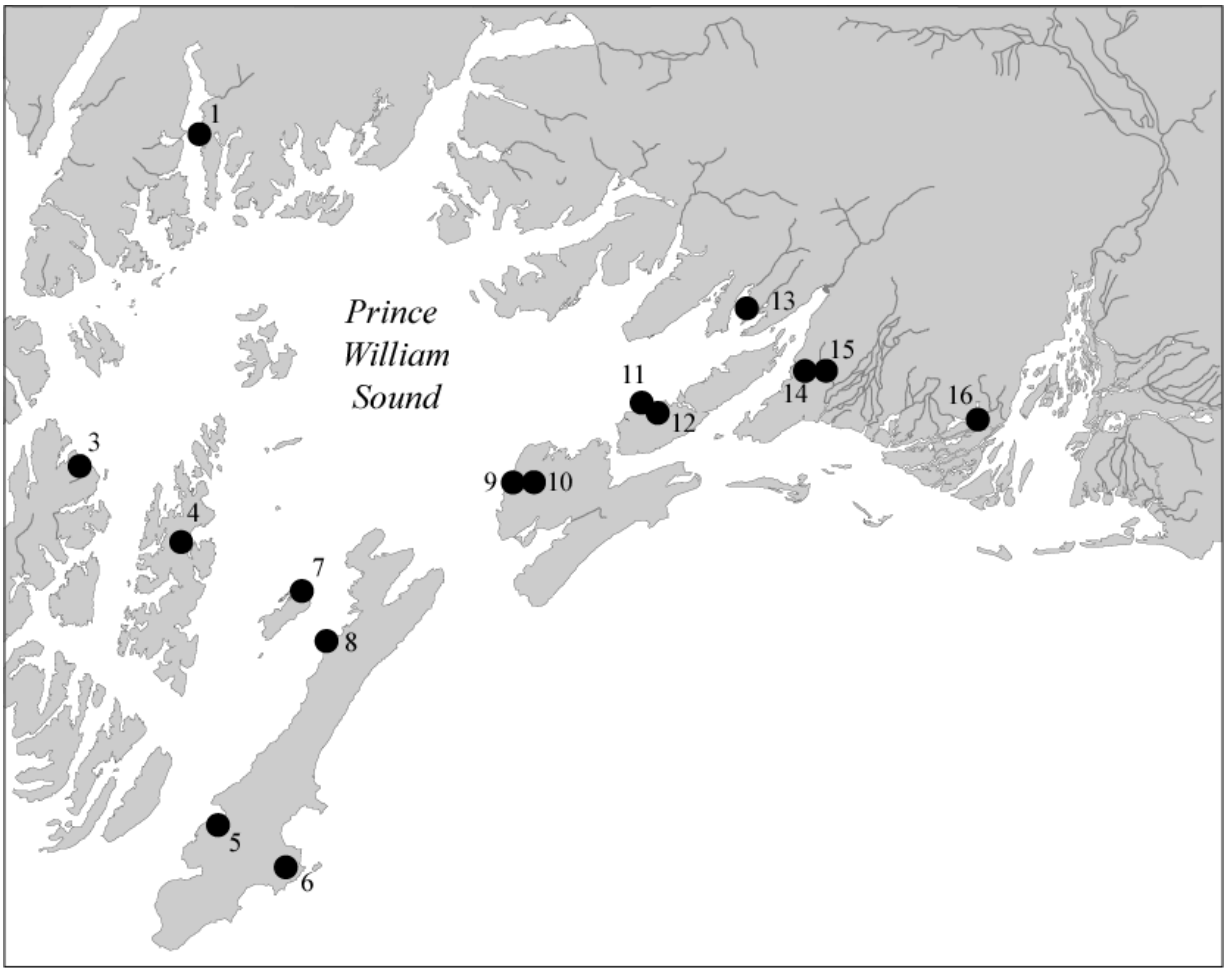


Figure 2. Sampling locations of Dolly Varden collected in 1996 and 1997 in Prince William Sound, Alaska. Codes are (1) Unakwik Inlet; (2) Shrode Lake; (3) Eshamy Bay; (4) West Arm, Bay of Isles; (5) Hanning Creek; (6) Stump Lake; (7) Green Island; (8) Port Chalmers; (9) Shelter Bay-below barrier; (10) Shelter Bay-above barrier; (11) Hawkins Creek-below barrier; (12) Hawkins Creek-above barrier; (13) Milton Lake; (14) Power Creek-below barrier; (15) Power Creek-above barrier; and (16) Clear Creek.

Table 2. Location, sample size, latitude and longitude, and natal habitat classification of 1996 and 1997 collections of Dolly Varden in Prince William Sound, Alaska. Natal habitats are classifications used for tests of population structure associated ecological differences. Island habitat refers to locations in the Prince William Sound Islands ecosection (see text for details). Site number refers to location in Figure 2. ** denotes sites used for mtDNA analysis.

	Sample Size		Location			
Name (site number)	(1996)	(1997)	Lat.	Lon.	Natal Habitats	
** Unakwik Inlet (1)	19	40	61.014	147.485	West	Mainland
Shrode Lake (2)	0	37	60.685	148.259	West	Mainland
** Eshamy Bay (3)	18	38	60.462	148.054	West	Mainland
Knight Island						
** West Arm, Bay of Isles (4)	41	25	60.388	147.715	West	Island
Montague Island						
** Hanning Creek (5)	40	32	59.970	147.653	West	Island
Stump Lake (6)	40	37	59.868	147.488	West	Island
Green Island (7)	13	0	60.283	147.400	West	Island
Port Chalmers (8)	0	19	60.244	147.202	West	Island
Hinchinbrook Island						
** Shelter Bay, below barrier (9)	40	40	60.429	146.652	East	Island
Shelter Bay, above barrier (10)	0	29	60.412	146.578	East	Island
Hawkins Island						
** Hawkins Creek, below barrier (11)	32	22	60.515	146.206	East	Island
** Hawkins Creek, above barrier (12)	31	28	60.479	146.142	East	Island
Milton Lake (13)	0	37	60.644	145.802	East	Mainland
** Power Creek, below barrier (14)	32	0	60.583	145.622	East	Island
** Power Creek, above barrier (15)	23	0	60.611	145.509	East	Island
**Clear Creek (16)	22	25	60.559	144.749	East	Mainland

Table 3. Enzymes and loci examined with starch gel electrophoresis in coastal cutthroat trout. Enzyme names are from the International Union of Biochemistry (IUB). Tissues were: M - Muscle, L - liver, E - Eye, and H - Heart. Buffer systems were: TBCLE - a Tris-citrate gel buffer and lithium hydroxide borate tray buffer (Ridgway et al. 1970); TG – 3.0 g/L Tris and 14.4 g/L glycene, ph 8.5; and ACE - an amine-citrate-EDTA gel and tray buffer (Clayton and Tretiak 1972).

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Aspartate aminotranferase	*mAAT-1**	E	ACE
(2.6.1.1)	*mAAT-2**	E	ACE
	*sAAT-1,2**	M	ACE
	*sAAT-3**	E	ACE.
Alcohol dehydrogenase	*ADH**	L	TBCLE
(1.1.1.1)			
Adenylate kinase	*AK-1**	E	ACE
(2.7.4.3)	*AK-2**	E	ACE
Aconitate hydratase	*sAH**	L	ACE
(4.2.1.3)			
Creatine kinase	*CK-A1**	M	TBCLE
(2.7.3.2)	*CK-A2**	M	TBCLE
	*CK-B**	E	ACE
	*CK-C1**	E	ACE
	*CK-C2**	E	ACE
Fructose-biphosphate aldolase	*FBALD-1**	E	TG
(4.1.1.13)	*FBALD-2**	E	TG
Glyceraldehyde-3-phosphate	*GAPDH-2**	H	ACE
dyhydrogenase	*GAPDH-3**	H	ACE
(1.2.1.12)	*GAPDH-4**	H	ACE
	*GAPDH-5**	H	ACE
Guanine deminase	*GDA-1**	L	TBCLE
(3.5.4.3)	*GDA-2**	L	TBCLE
Glycerol-3-phosphate	*G3PDH-1**	M	ACE
dehydrogenase (1.1.1.8)	*G3PDH-2**	M	ACE

Table 3. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Glucose-6-phosphate isomerase (5.3.1.9)	*GPI-A**	M	TBCLE
	*GPI-B1**	M	TBCLE
	*GPI-B2**	M	TBCLE
Isocitrate dehydrogenase ($NADP^+$) (*1.1.142*)	*sIDH-1,2**	H, L	ACE
	*mIDHP-1**	H	ACE
	*mIDHP-2**	H	ACE
l-Lactate dehydrogenase (1.1.1.27)	*LDH-A1**	M	TBCLE
	*LDH-A2**	M	TBCLE
	*LDH-B1**	E	TG
	*LDH-B2**	E, L	TBCLE
	*LDH-C**	E	TG
Malate dehydrogenase (1.1.1.37)	*sMDHA-1,2**	H, L	ACE
	*sMDHB-1,2**	H, L	ACE
Malic enzyme (1.1.1.40)	*MEP-1**	M	ACE
	*sMEP-1**	M	ACE
	*sMEP-2**	L	ACE
Dipeptidase (3.4.13.18)	*PEP-A**	M	TG
Proline dipeptidase (3.4.13.9)	*PEP-D**	E	ACE
Phosphogluconate dehydrogenase (1.1.1.44)	*PGDH**	M	ACE
Phosphoglucomutase (5.4.2.2)	*PGM-1**	L	ACE
	*PGM-2**	L	ACE
Superoxide dismutase (1.15.1.1)	*sSOD-1**	L	TBCLE
Triose-phosphate isomerase (5.3.1.1)	*TPI-1**	M	TG
	*TPI-2**	M	TG
	*TPI-3**	E	ACE
	*TPI-4**	E	ACE

Table 4. Enzymes and loci examined with starch gel electrophoresis in Dolly Varden. Enzyme names are from the International Union of Biochemistry (IUB). Tissues were: M – muscle, L - liver, E - eye, and H - heart. Buffer systems: TG – 3.0 g/L tris and 14.4 g/L glycene, pH 8.5; TBCLE - a Tris-citrate gel buffer pH 8.2 and lithium hydroxide borate acid tray buffer (Ridgeway et al. 1970), ACE - an amine-citrate-EDTA gel and tray buffer pH 6.8 (Clayton and Tretiak 1972) ACE 6.1 - a citrate amine pH 6.1., and TC4 – 27 g/L tris and 18.1 g/L citric acid, pH 5.95. All gel buffers tested are shown.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Aspartate aminotranferase (2.6.1.1)	*sAAT-1,2**	M,H	TBCLE, ACE 6.8, AC 6.1, TC4
	*sAAT-3**	E	TG, ACE 6.8
	*sAAT-4**	L	TBCLE, ACE 6.8
Adenosine deaminase (3.5.4.4)	*ADA-1**	M	TG, ACE 6.8
	*ADA-2**	M	TG, ACE 6.8
Alcohol dehydrogenase (1.1.1.1)	*ADH**	L	TBCLE, ACE 6.8
Aconitate hydratase (4.2.1.3)	*sAH**	L	TBCLE
	*mAH-1**	M,E,H	ACE 6.8, TC4
	*mAH-2**	M,E,H	ACE 6.8, TC4
Alanine aminotransferase (2.6.1.2)	*ALAT**	M	TG
Creatine kinase (2.7.3.2)	*CK-A1**	M	TG
	*CK-A2**	M	TG
Esterase-D (methylumbelliferyl) (3.1.1.*)	*ESTD**	M	TBCLE
Formaldehyde dehydrogenase (1.2.1.1)	*FDHG**	M,L	TG
Fumerate hydratase (4.2.1.2)	*FH**	M,L,E,H	ACE 6.8, AC 6.1, TC4
N-Acetyl-b-glucosaminidase (3.2.1.52)	*bGLUA**	L	TBCLE, ACE 6.8

Table 4. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Glucose-6-phosphate isomerase (5.3.1.9)	*GPI-A**	M,E	TG, TBCLE
	*GPI-B1**	M	TG, TBCLE
	*GPI-B2**	M	TG, TBCLE
Glutathione reductase (1.6.4.2)	*GR**	M,E	ACE 6.8, TG, AC 6.1
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	*G3PDH-1**	M,L,H	TBCLE, AC 6.1, ACE 6.8
	*G3PDH-2**	M,L,H	TBCLE, AC 6.1, ACE 6.8
l-Iditol 2-dehydrogenase (1.1.1.14)	*IDDH-1**	L	TBCLE, TG
	*IDDH-2**	L	TBCLE, TG
Isocitrate dehydrogenase (NADP) (1.1.1.42)	*mIDHP-1**	M,H	ACE 6.8
	*mIDHP-2**	M,H	ACE 6.8
	*sIDHP-1,2**	M,L,E,H	ACE 6.8, AC 6.1, TC4
l-Lactate dehydrogenase (1.1.1.27)	*LDH-A1**	M	TBCLE
	*LDH-A2**	M	TBCLE
	*LDH-B1**	M,E	TBLCE, TG, ACE 6.8
	*LDH-B2**	M,E	TBLCE, TG, ACE 6.8
	*LDH-C**	E	TG
Malate dehydrogenase (1.1.1.37)	*sMDHA-1,2**	L,E,H	ACE 6.8, AC 6.1, TC4
	*sMDHB-1,2**	M,H	ACE 6.8, AC 6.1, TC4
Mannose-6-phosphate isomerase (5.3.1.8)	*MPI**	M,L,E	TG
Nucleoside-trisphosphate pyrophosphate (3.6.1.19)	*NTP**	M	TBCLE
Dipeptidase (3.4.13.18)	*PEP-A**	M,L,E	TBCLE, TG
Tripeptide aminopeptidase (3.4.11.4)	*PEP-B1**	M,L,E,H	TBCLE, TG
Proline dipeptidase (3.4.13.9)	*PEP-D1**	M,L,H	ACE 6.8, AC 6.1, TC4
	*PEP-D2**	M,H	ACE 6.8,

Table 4. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Leucyl-l-tyrosine peptidase (3.4.-.-)	*PEP-LT**	M,E,H	TG, TC4
Phosphogluconate dehydrogenase (1.1.1.44)	*PDGH**	M,L,E	ACE 6.8
Phosphoglycerate kinase (2.7.2.3)	*PGK-1**	M,H	ACE 6.8
	*PGK-2**	M,H	ACE 6.8
Phosphoglucomutase (5.4.2.2)	*PGM-1**	M,L	ACE 6.8 ,TG
	*PGM-2**	M,L	ACE 6.8 ,TG, AC 6.1
Superoxide dismutase (1.15.1.1)	*sSOD-1**	M,L,H	TBCLE, TG
Triose-phosphate isomerase (5.3.1.1)	*TPI-1**	E	TG
	*TPI-2**	E	TG
	*TPI-3**	E	TG
	*TPI-4**	E	TG

individual plastic tubes labeled with a unique identification number, and frozen on dry ice. Fish <250 mm were frozen whole. Where only non-lethal fin clips were taken, a fish were anesthetized and approximately 3 mm^2 of the caudal fin was removed from each fish, placed on dry ice or in 95% ethanol, and the fish released. All frozen samples were stored in an -80^o C freezer at the ADFG office in Cordova, Alaska, until they were transported on dry ice to the Oregon Cooperative Fish Research Unit (OCFRU) laboratory in Corvallis, Oregon. There, tissues from whole fish were removed and transferred to 1.7 ml microcentrifuge tubes while frozen to be stored at -80^o C.

Laboratory Analyses

Allozyme analysis of coastal cutthroat trout and Dolly Varden followed methods of Aebersold et al. (1987). We examined variation at 48 loci in coastal cutthroat trout (Table 3) and 51 loci in Dolly Varden (Table 4). Allele designations for coastal cutthroat trout were relative to the mobility of the common allele in coastal rainbow trout. Dolly Varden analyses were conducted at the Washington Department of Fish and Wildlife Genetics Laboratory.

DNA was extracted from muscle or caudal fin tissue using a phenol:choroform:isoamyl alcohol extraction procedure, suspended in TE buffer (Sambrook et al. 1989), and stored at 2° C for mitochondrial DNA and microsatellite DNA analyses. Analysis of restriction-fragment length-polymorphisms (RFLP) in mitochondrial DNA of 80 coastal cutthroat trout from eight locations and 94 Dolly Varden from 10 locations followed methods of Cronin et al. (1993). We amplified three mitochondrial DNA subunits, NADH dehydrogenase-1 (ND-1), ND-2, and control region (D-loop) segments using polymerase chain reaction (PCR) and primers developed by LGL Genetics, Inc. Amplification reactions were conducted in 50µl volumes, consisting of 1-5.0 µL extracted DNA, 5.0µL LGL *Taq* buffer, 1.0µL forward primer, 1.0µL reverse primer, 0.4µL dNTP (0.1µLeach), 0.5µL *Taq* polymerase, and 37.1-41.1 µlL pure water (LGL Genetics, Inc.). Subunit ND-1 was digested with nine restriction enzymes, *Alu I*, *Ava II*, *Bgl II*, *Dpn II*, *Hae III*, *Hind III*, *Msp I*, *Taq I*, *BstU I*; ND-2 was digested with three restriction enzymes, *Alu I*, *Hind III*, *Mse I*; and D-loop was digested with four restriction enzymes, *Bgl II*, *Dpn II*, *Hha I*, *Mse I*, based on experience with other cutthroat trout species (P. Evans, Brigham Young University, personal communication) and other salmonids. DNA fragments were separated by electrophoresis on 2.3 % agarose gels and stained with ethidium bromide. Fragment patterns were photographed under ultraviolet light. Restriction fragment sizes were estimated using a 1-kilobase ladder as a molecular weight reference. Restriction fragment patterns produced by each of the mtDNA subunit-restriction enzyme combinations were used to defined composite haploytpes (Lansman et al. 1981).

We examined 13 microsatellite primers (Table 5) for coastal cutthroat trout and Dolly Varden, respectively, following methods of Wenburg et al. (1996), optimized to conditions in our laboratory. Primer sequences and sources were described in Olson et al. (1996). $MgCl_2$ concentrations, primer concentration, and annealing temperature for polymerase chain reactions (PCR) were varied experimentally to minimize stutter bands that make microsatellite variation difficult to interpret (Wenburg et al. 1996). All PCR reactions, except for Set 3 primers, used 20 µL volumes of total reaction cocktail. These consisted of an amplification reaction cocktail (2 µL 10X buffer, 0.04 µL of each of the four dNTP's, 2.0 µL $MgCl_2$, 0.2 µL Amplitaq gold, and 6.64 µL water), a primer cocktail of primer (see Table 5 for concentrations) and water up to 8 µL

Table 5. Microsatellite primers, multiplexing and amplification protocols for coastal cutthroat trout and Dolly Varden collected from Prince William Sound, Alaska. See Olson et al. (1996) for primer sequences, references, and source species.

Multiplex Set	Primer	Forward primer label	Concentration	Annealing Temperature
Set 1a	Sfo8	Fam	0.20µM	56 ° C
	Ssa85	Tet	0.30µM	56 ° C
	Omy77	Hex	0.15µM	56 ° C
Set 1b	Oneµ11	Fam	0.10µM	56 ° C
	Ots1	Tet	0.50µM	56 ° C
	Oneµ14	Hex	0.40µM	56 ° C
Set 2	Oneµ2	Fam	0.60µM	52 ° C
	Ssa14	Tet	0.55µM	52 ° C
	Omy325	Hex	0.40µM	52 ° C
Set 3	Ots4	Fam	0.50µM	52 ° C
	Oneµ8	Tet	0.80µ M	52 ° C
	Sfo12	Fam	0.50µ M	52 ° C
	Sfo23	Tet	0.50µM	52 ° C

total volume, and 1 μL DNA. For primers from multiplex set for cutthroat trout, volume of $MgCl_2$ was increased to 2.4 μL and water was reduced to 4.24 μL in the amplification cocktail and the primer cocktail volume was 10 μl/ sample. Amplification reactions for PCR products were separated on a denatured 6% polyacrylamide gel using a Perkin Elmer Applied Biosystems, Inc. (ABI) 377 automated sequencer and analyzed using ABI GeneScan 672, analysis software, version 2.0.2. A tamra-350 (Abi Prism Gene Scan^{TM}) 350 internal lane standard was used for each individual sample. Genotypes were scored from pherograms with basepair sizing generated by Genotyper software. Amplified product from one individual was run on every gel to determine if runs between gels were consistent. In addition, approximately 5% of the total sample was rerun to ensure that results were consistent. Primers that gave trouble-free resolution were chosen for further analysis.

Fish for meristic analysis were randomly selected from five collections of coastal cutthroat trout from different geographic areas of PWS, fixed in 10% formalin, and stored in 70% isopropanol. Counts were made on five meristic characters: (1) scales above the lateral line (dorsal scale rows); (2) scales in the lateral line series; (3) pelvic fin rays; (4) branchiostegal rays; and (5) gill rakers on the upper and lower limb of the gill arch. Bilateral traits were counted on the left side of the fish. Analysis of variance was used to test for statistical differences among groups. Means plots were constructed and multiple comparison tests were conducted using Fisher's least significant difference (LSD) method (Sokal and Rohlf 1995).

We examined differences in otolith microchemisty in Dolly Varden and coastal cutthroat trout above and below barriers in Power Creek and Shelter Bay, respectively. For the Dolly Varden, our objective was to test the ability of otolith microchemistry to identify those with fresh or salt water histories, as this technique had not been tried for this species. Consequently, Dolly Varden were from above and below a 40 m waterfall on Power Creek that provided a complete barrier to migration of amphidromous Dolly Varden into the resident population. For coastal cutthroat trout our objective was to test whether the presumed barrier on Shelter Creek was a true barrier to upstream migration and compare the results to genetic analyses.

Sagittal otoliths were removed from each fish and stored separately in dry microcentrifuge tubes until they could be prepared for analysis. Each otolith was then mounted sulcus side down with a heat sensitive bonding agent (Crystal Bond) on a glass cover slip and attached by a single corner to a microscope slide. With progressively finer grits of sandpaper, the otolith was ground on one side, heated to release the bonding agent, and remounted to expose the other side for grinding until the nuclear region of the otolith was exposed, as revealed under a light microscope. The otolith was then polished with a paste of 0.05 μm alumina powder mixed with water and remounted to a petrographic slide for electron microprobe analysis. Elemental analysis of otoliths was conducted with a Cameca SX-50 wavelength dispersive electron microprobe located at Oregon State University along a transect from the primordial region, which has been shown to reflect maternal inheritance, to the edge of the otolith. Methods for counting time and beam diameter follow those of Toole and Nielsen (1992). Srontiantite and calcite were used as standards for Sr and Ca. We used paired t-tests to test for difference in the mean Sr/Ca in primordial between fish above and below barriers.

Genetic Data Analysis

The history of post-glacial colonization, bottlenecks in population abundance, and existing migratory patterns of coastal cutthroat trout and Dolly Varden affect patterns of genetic diversity in different spawning aggregations. We examined genetic diversity of cutthroat trout and Dolly Varden in PWS from different collection locations by estimating expected average heterozygosity, Nei's unbiased heterozygosity (Nei 1987), and number of alleles per locus for allozyme and microsatellite DNA variation, and nucleotide substitutions per site ($\hat{d}$;Nei 1987) and nucleotide diversity (π; Nei 1987) for mitochondrial DNA polymorphisms. We tested genotypic proportions for deviations from Hardy-Weinberg equilibrium using the Markov chain exact test (Guo and Thompson 1992) in GENEPOP version 3.3. We tested for differences among loci in deviations from Hardy-Weinberg proportions using Kendall's concordance test (Sokal and Rohlf 1995) of F_{IS} values using 10,000 Monte Carlo iterations in StatXact 4. Because single-locus heterozygosities among closely related populations are historically correlated, we tested for differences in average unbiased heterozygosity (H_e) among different locations using a paired Student's t-test over all polymorphic loci, following recommendations of Nei (1987). Because the number of alleles per locus and heterozygosity depend on the sample size, we estimated mean number of alleles per locus, H_e, and the 95% confidence intervals by resampling without replacement an equal number of individuals 1,000 times at each locus (but with replacement for different loci) using POPTOOLS (a Microsoft Excel add-in available from Greg Hood, Commonwealth Scientific and Industrial Research Organization, Australia at www.cse.csiro.au/CDG/poptools). We tested for differences in genotypic frequencies between years within sampling locations and for differences between populations above and below barriers using the log-likelihood (G) Monte Carlo-based exact tests (Raymond and Rousset 1995) available in GENEPOP 3.3 and StatXact 4. We corrected for multiple comparisons using sequential Bonferroni corrections (Rice 1989).

To examine geographical patterns of genetic similarity suggested by the data we constructed dendrograms based on cluster analysis of pairwise genetic distances among locations using the unweighted pair-group method with arithmetic averages (UPGMA) clustering algorithm (Sneath and Sokal 1973). We used coancestry distance (Weir 1996) for allozyme and microsatellite DNA data and average nucleotide divergence between populations (Nei 1987) for mitochondrial DNA data. For Dolly Varden, the analysis was based on the combined allozyme and microsatellite DNA data.

The life-history of coastal cutthroat trout and Dolly Varden and differences in freshwater habitat across Prince William Sound suggested that genetic population structure might be associated with ecological differences in natal habitats. We tested two hypotheses of population structure using the 95% confidence intervals for the F-statistic for differentiation among regions (θ_P) and among local aggregations with regions (θ_S) for a three-level population hierarchy. The 95% confidence intervals were obtaining by bootstrapping over loci 10,000 times using GDA (by Paul Lewis and Dmitri Zaykin, http://lewis.eeb.uconn.edu/lewishome/software.html). The first hypothesis was that population structure might reflect differences in spawning substrates associated with east-west differences. Streams east of Montague Island are composed largely of sand and gravel from the Copper River and near-coastal piedmont glaciers, whereas the coastline to the west is largely rocky (Mann and Hamilton 1995). Populations in the two regions are

separated by a large, deep-water channel from the Gulf of Alaska that tends towards the northwest between Montague and Hinchinbrook islands. The second hypothesis was that population structure might reflect environmental differences associated with ecoregional differences. We classified populations for this analysis using ecosection analyses and maps developed by the USDA Forest Service (Davidson 1997). Coastal cutthroat trout and Dolly Varden in the Prince William Sound Islands ecosubsection, except the mainland collection from Eshamy Bay, were considered island populations, and fish from the Lowe and Copper rivers ecosubsections were considered mainland populations (Tables 1, 2).

Population structure in PWS coastal cutthroat trout and Dolly Varden might also reflect isolation by distance. We tested three modes of dispersal under isolation-by-distance hypotheses. First, dispersal might follow the possible post-glacial colonization route from east to west along the outer island around the circumference of PWS. This kind of configuration approximately corresponds to a one-dimensional stepping stone model for dispersal (Kimura and Weiss 1964). We also tested for isolation by distance assuming dispersal along the shortest possible shoreline distance between locations, which allowed for dispersal along the mainland shoreline. In both cases, we assumed that coastal cutthroat could cross mouths of inlets (5-10 km). Finally, we tested for isolation by distance, assuming the shortest possible distance between locations, which allowed coastal cutthroat trout and Dolly Varden to navigate long distances of open, deep water. If such dispersal were the general case, gene flow might approximate a two-dimensional stepping stone model. We measured distances between locations under these three hypotheses using Delorme Topo USA 4.0 at 1:400,000. Distances between populations above and below barriers in the same stream were arbitrarily set to 5 km. We calculated pairwise values of θ and tested for correlation between θ and $\log_{10}$ transformed geographical distance, d, with Mantel tests with 10,000 randomizations using GENEPOP 3.3. We calculated the number of pairwise migrants, $\hat{M}$, following Slatkin (1991, 1993) and regressed $\hat{M}$ on $\log_{10}(d)$ using ordinary least squares (OLS) regression. To examine the effect of spatial scale on isolation by distance we regressed the slope of $\hat{M}$ on $\log_{10}(d)$ for successive pairwise populations at increasing geographical distance.

Because amphidromous Dolly Varden and coastal cutthroat trout sometimes enter non-natal streams to forage and overwinter, collections of below barriers could potentially contain mixtures of different populations. This could lead to deviations from Hardy-Weinberg equilibrium, gametic disequilibrium from gene flow, and non-representative allele frequencies that confound other population structure analyses. To address this possibility, we used a Bayesian clustering approach (STRUCTURE version 2) that accounts for the presence of Hardy-Weinberg and linkage disequilibrium in the samples by assigning individuals to K population groupings that maximize within-group equilibrium (Pritchard et al. 2000). We included resident fish isolated above barriers in the analyses because of the possibility that they could contribute to downstream anadromous samples. We chose the smallest K that best explained the structure in the data by repeating the analysis with $K = 1$ to 8 and calculating the posterior probabilities for each analysis using Bayes's Rule, assuming uniform prior probabilities. Each analysis was based on 10^5 simulations after a burn-in of 30,000.

Table 6. Allozyme frequencies and sample sizes (N) for polymorphic loci in PWS coastal cutthroat trout. Collection site numbers refer to locations in Figure 1.

		Collection Location						
Locus	Allele	12	10	7	8	5	2	3
*AK-1**	N	17	17	4	28	17	20	22
	100	0.971	0.912	0.250	0.804	0.824	0.975	0.909
	37	0.029	0.088	0.750	0.196	0.176	0.025	0.091
*CK-C2**	N	18	18	15	30	16	16	23
	100	0.694	0.861	0.867	0.883	0.719	0.969	0.543
	109	0.194	0.139	0.133	0.067	0.000	0.031	0.457
	104	0.111	0.028	0.000	0.050	0.281	0.000	0.000
*GDA-1**	N	19	24	19	28	17	20	23
	100	0.816	1.000	1.000	0.929	0.941	0.900	0.978
	115	0.184	0.000	0.000	0.714	0.059	0.100	0.022
*GDA-2**	N	20	19	16	28	17	20	23
	100	0.725	0.395	0.313	0.768	0.588	0.650	0.630
	87	0.275	0.605	0.688	0.232	0.412	0.350	0.370
*sIDHP-1,2**	*N*	*80*	*100*	*72*	*120*	*76*	*80*	*92*
	100	0.225	0.300	0.292	0.250	0.263	0.250	0.250
	71	0.700	0.680	0.708	0.750	0.737	0.750	0.750
	22-28	0.075	0.020	0.000	0.000	0.000	0.000	0.000
*sMDHA-1,2**	N	76	92	68	120	80	92	76
	100	0.855	0.772	0.779	0.825	0.837	0.989	0.849
	43	0.145	0.228	0.221	0.175	0.162	0.011	0.151
*sMEP-1**	N	19	26	20	30	20	20	23
	100	0.921	1.000	1.000	1.000	1.000	1.000	1.000
	110	0.079	0.000	0.000	0.000	0.000	0.000	0.000
*sMEP-2**	N	20	26	20	30	20	20	23
	100	0.100	0.000	0.000	0.000	0.000	0.000	0.000
	114	0.900	1.000	1.000	1.000	1.000	1.000	1.000
*MPI**	N	20	26	20	30	20	20	23
	100	0.975	0.981	1.000	1.000	1.000	1.000	1.000
	95	0.025	0.096	0.000	0.000	0.000	0.000	0.000

Table 6. Continued.

		Collection Location						
Locus	Allele	12	10	7	8	5	2	3
*PEP-A**	N	20	26	20	30	20	14	18
	100	0.000	0.019	0.000	0.000	0.025	0.000	0.000
	112	1.000	0.981	1.000	1.000	0.975	1.000	1.000
*SOD-1**	N	19	25	20	29	20	20	23
	100	0.868	0.840	0.800	0.759	0.975	0.650	0.761
	142	0.132	0.140	0.150	0.224	0.025	0.350	0.239
	42	0.000	0.020	0.050	0.017	0.000	0.000	0.000

Results

Coastal Cutthroat Trout

We hypothesized that the dynamics of extinction and colonization in cutthroat trout at the northern edge of the species range would influence patterns of within-population genetic diversity. Genetic diversity in PWS coastal cutthroat trout was lower than we expected. All individuals of the 80 individuals from eight locations throughout PWS, except two individuals, had identical mtDNA genotypes. These individuals were characterized by polymorphisms in the D-loop region when restricted with *Dpn II* and *Hha I*. Restrictions with *Dpn-II* yielded two basepair (bp) fragment-size patterns: (A) 850-, 530-bp and (B) 850-, 650-bp. *Hha I* also yielded two patterns: (A) 800-, 450-bp and (B) 800-, 510-bp. In both cases fragments under 100 base pairs were not resolved. Nucleotide substitutions per site, $\hat{d}$, between haplotypes was 0.0028. Only 11 of 40 allozyme loci were polymorphic (Table 6) and with an average of 1.15 alleles per locus (Figure 3). Nei's unbiased average heterozygosity (H_e) ranged from 0.028-0.050 (Figure 4). All five microsatellite loci that we successfully resolved were polymorphic (Table 7) but they had mean number of alleles per locus across 13 populations that ranged from 2.51-10.24 (Figure 3). H_e for microsatellite DNA variation ranged from 0.368-0.842 (Figure 4).

Distribution of within-location genetic diversity showed a strong geographical pattern. Coastal cutthroat trout from more remote northwestern locations in PWS had significantly fewer alleles per locus and significantly lower H_e at microsatellite loci than cutthroat trout below barriers in other areas of PWS. Cutthroat trout from Columbia Bay, Unakwik Inlet, Gunboat Lakes, West Arm, and Green Island averaged 2.51-6.35 alleles per locus, whereas cutthroat trout below barriers in other areas of PWS averaged 8.68-10.24 alleles per locus (Figure 3a). Cutthroat trout isolated by barriers in Hawkins Creek had an intermediate number of alleles per locus (7.75), as did cutthroat trout from Eighteenmile Creek in the Copper River delta (7.07). A similar pattern existed for at H_e microsatellite loci. Cutthroat trout from Columbia Bay, Unakwik Inlet, Gunboat Lakes, West Arm, and Green Island had lower H_e, ranging from 0.368-0.712, whereas H_e for cutthroat from other locations were higher, ranging from 0.804-0.842 (Figure 4a). The Columbia Bay population, which is the most northern known population of coastal cutthroat trout, had the lowest H_e, lowest number of alleles per locus, and was nearly fixed at *Sfo8* (H_e = 0.053) and *Omy325* (H_e = 0.092), although the loci were highly variable in other populations (median H_e =0.834 and H_e =0.827, respectively). We detected no significant differences in mean number of alleles per locus for cutthroat trout above and below putative barriers or at allozyme loci.

We also hypothesized that geographical genetic structure in coastal cutthroat trout in PWS might be associated with different geographical regions. Neither the cluster analyses nor tests of parapatric differentiation associated with natal habitat supported this hypothesis. Cluster analysis for both allozyme and microsatellite DNA data showed no evidence of discrete geographical genetic groups. Cluster analysis of microsatellite DNA differences among coastal cutthroat trout from different locations, for example, detected a genetically similar group of cutthroat trout from locations around Orca Bay (Milton Lake and locations on Hinchinbrook and Hawkins Island) with increasing stepwise genetic divergence of cutthroat trout from more distant

Table 7. Microsatellite DNA frequencies and sample sizes (N) in PWS coastal cutthroat trout. Collection locations refer to locations in Figure 1.

		Collection Location												
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13
Omy325	N	21	31	36	15	36	16	27	41	16	31	26	26	17
	98	0.000	0.032	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000
	104	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.032	0.000	0.000	0.000
	106	0.048	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.016	0.000	0.000	0.059
	110	0.952	0.032	0.000	0.000	0.194	0.031	0.185	0.122	0.031	0.161	0.000	0.096	0.029
	112	0.000	0.000	0.000	0.000	0.000	0.938	0.000	0.000	0.000	0.000	0.000	0.000	0.088
	114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
	118	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.147
	120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
	136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000
	140	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	144	0.000	0.000	0.208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000
	146	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.250	0.000	0.000	0.000	0.000
	148	0.000	0.065	0.722	0.067	0.000	0.000	0.167	0.110	0.063	0.387	0.385	0.173	0.118
	150	0.000	0.065	0.000	0.000	0.083	0.000	0.056	0.110	0.094	0.000	0.019	0.019	0.059
	152	0.000	0.097	0.000	0.100	0.208	0.000	0.056	0.110	0.000	0.016	0.000	0.058	0.000
	154	0.000	0.016	0.000	0.000	0.014	0.000	0.074	0.073	0.063	0.000	0.000	0.096	0.000
	156	0.000	0.016	0.000	0.000	0.014	0.000	0.019	0.000	0.031	0.032	0.000	0.019	0.353
	160	0.000	0.016	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.016	0.000	0.000	0.000
	162	0.000	0.113	0.000	0.000	0.000	0.000	0.074	0.012	0.000	0.000	0.077	0.000	0.000
	164	0.000	0.355	0.069	0.133	0.000	0.000	0.074	0.110	0.188	0.129	0.135	0.077	0.000

Table 7. Continued.

		Collection Location												
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13
Omy325	166	0.000	0.016	0.000	0.300	0.000	0.000	0.037	0.024	0.063	0.065	0.077	0.096	0.000
	168	0.000	0.032	0.000	0.167	0.042	0.031	0.000	0.098	0.156	0.097	0.173	0.154	0.147
	170	0.000	0.000	0.000	0.133	0.042	0.000	0.074	0.012	0.000	0.000	0.000	0.038	0.000
	172	0.000	0.000	0.000	0.033	0.000	0.000	0.019	0.012	0.000	0.000	0.000	0.038	0.000
	174	0.000	0.000	0.000	0.000	0.014	0.000	0.019	0.024	0.000	0.000	0.000	0.000	0.000
	176	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.024	0.000	0.032	0.019	0.038	0.000
	178	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.016	0.000	0.019	0.000
	180	0.000	0.016	0.000	0.067	0.014	0.000	0.000	0.012	0.000	0.000	0.000	0.077	0.000
	184	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.037	0.031	0.000	0.000	0.000	0.000
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
	188	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	190	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	192	0.000	0.065	0.000	0.000	0.000	0.000	0.037	0.012	0.000	0.000	0.000	0.000	0.000
Ssa14	N	16	21	36	14	21	7	31	35	17	30	23	31	17
	106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000
	110	0.000	0.000	0.000	0.000	0.024	0.143	0.000	0.043	0.000	0.000	0.000	0.000	0.000
	112	0.000	0.048	0.000	0.000	0.071	0.143	0.000	0.014	0.000	0.000	0.000	0.000	0.147
	114	0.000	0.000	0.000	0.000	0.000	0.286	0.000	0.057	0.000	0.000	0.000	0.000	0.029
	116	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	118	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000
	120	0.000	0.000	0.000	0.000	0.095	0.000	0.016	0.029	0.059	0.050	0.000	0.016	0.000
	122	0.000	0.000	0.000	0.000	0.286	0.000	0.000	0.057	0.000	0.000	0.000	0.000	0.000
	124	0.688	0.429	0.514	0.393	0.190	0.286	0.516	0.486	0.441	0.350	0.261	0.452	0.176
	126	0.313	0.524	0.347	0.214	0.071	0.071	0.290	0.171	0.147	0.350	0.391	0.403	0.059

Table 7. Continued.

		Collection Location												
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13
Ssa14	128	0.000	0.000	0.028	0.071	0.000	0.000	0.016	0.029	0.059	0.033	0.109	0.016	0.000
	130	0.000	0.000	0.014	0.036	0.000	0.000	0.016	0.014	0.029	0.017	0.130	0.000	0.235
	132	0.000	0.000	0.000	0.179	0.048	0.071	0.113	0.000	0.118	0.017	0.043	0.065	0.324
	134	0.000	0.000	0.056	0.107	0.119	0.000	0.000	0.029	0.118	0.150	0.065	0.016	0.000
	136	0.000	0.000	0.042	0.000	0.048	0.000	0.016	0.000	0.000	0.017	0.000	0.000	0.029
	138	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.029	0.017	0.000	0.032	0.000
Oneu2	N	5	35	35	14	33	12	28	39	14	27	25	25	17
	197	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	205	0.000	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000
	207	0.000	0.000	0.000	0.000	0.015	0.125	0.000	0.051	0.000	0.037	0.000	0.000	0.000
	209	0.200	0.014	0.514	0.000	0.061	0.083	0.196	0.064	0.143	0.148	0.080	0.340	0.000
	211	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.037	0.000	0.000	0.059
	213	0.000	0.243	0.029	0.179	0.136	0.042	0.179	0.154	0.179	0.204	0.340	0.160	0.294
	215	0.000	0.400	0.014	0.000	0.045	0.042	0.107	0.128	0.036	0.074	0.000	0.000	0.294
	217	0.000	0.029	0.000	0.000	0.000	0.000	0.036	0.026	0.000	0.000	0.000	0.000	0.118
	219	0.000	0.000	0.000	0.000	0.121	0.208	0.071	0.141	0.143	0.167	0.140	0.160	0.000
	221	0.200	0.157	0.000	0.000	0.364	0.083	0.286	0.192	0.179	0.130	0.100	0.200	0.118
	223	0.000	0.043	0.000	0.179	0.152	0.333	0.036	0.026	0.036	0.000	0.000	0.040	0.059
	225	0.000	0.000	0.000	0.000	0.106	0.042	0.018	0.026	0.000	0.000	0.080	0.000	0.000
	227	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	229	0.200	0.029	0.243	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000
	231	0.000	0.000	0.029	0.071	0.000	0.000	0.036	0.026	0.000	0.037	0.000	0.000	0.000
	233	0.400	0.029	0.014	0.571	0.000	0.000	0.000	0.090	0.250	0.130	0.260	0.100	0.059
	237	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	239	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	249	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.013	0.000	0.000	0.000	0.000	0.000

Table 7. Continued.

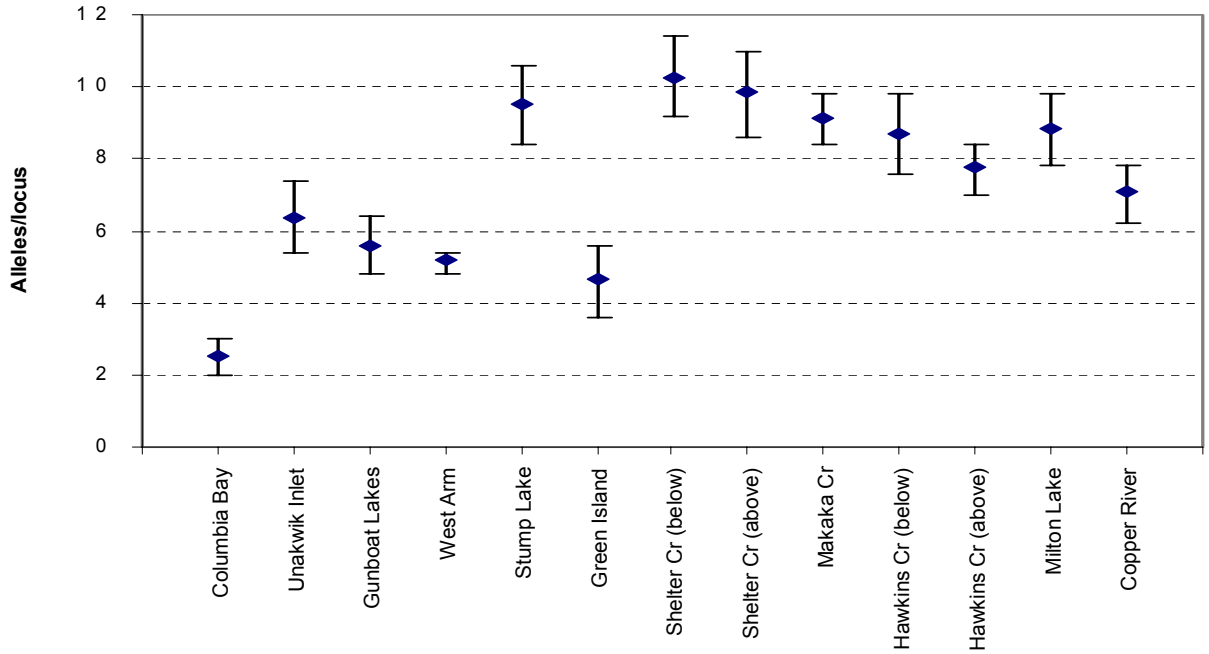
		Collection Location												
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13
Oneu2	253	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
Sfo8	N	19	33	33	13	36	7	31	43	19	34	37	37	17
	187	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	189	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	193	0.000	0.030	0.000	0.000	0.014	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000
	195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.027	0.000
	197	0.000	0.864	0.197	0.000	0.069	0.286	0.210	0.279	0.211	0.382	0.203	0.108	0.206
	199	0.000	0.000	0.015	0.000	0.000	0.143	0.016	0.000	0.026	0.000	0.000	0.014	0.000
	201	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000
	203	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000
	205	0.000	0.015	0.076	0.000	0.014	0.000	0.113	0.058	0.184	0.059	0.041	0.122	0.294
	207	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.029
	209	0.000	0.000	0.015	0.000	0.056	0.000	0.081	0.035	0.000	0.044	0.149	0.162	0.059
	211	0.974	0.000	0.167	0.538	0.361	0.214	0.323	0.221	0.289	0.294	0.378	0.257	0.265
	213	0.000	0.000	0.076	0.000	0.083	0.143	0.129	0.105	0.105	0.015	0.041	0.041	0.000
	215	0.000	0.000	0.015	0.000	0.014	0.071	0.000	0.012	0.000	0.015	0.000	0.027	0.000
	217	0.026	0.000	0.106	0.000	0.069	0.143	0.048	0.256	0.105	0.118	0.095	0.162	0.000
	218	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	219	0.000	0.000	0.045	0.000	0.014	0.000	0.016	0.000	0.000	0.000	0.014	0.041	0.000
	221	0.000	0.000	0.288	0.000	0.014	0.000	0.016	0.000	0.000	0.015	0.000	0.000	0.000
	223	0.000	0.000	0.000	0.000	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.088
	225	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059
	227	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	229	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	235	0.000	0.000	0.000	0.346	0.014	0.000	0.016	0.000	0.026	0.000	0.014	0.041	0.000

Table 7. Continued.

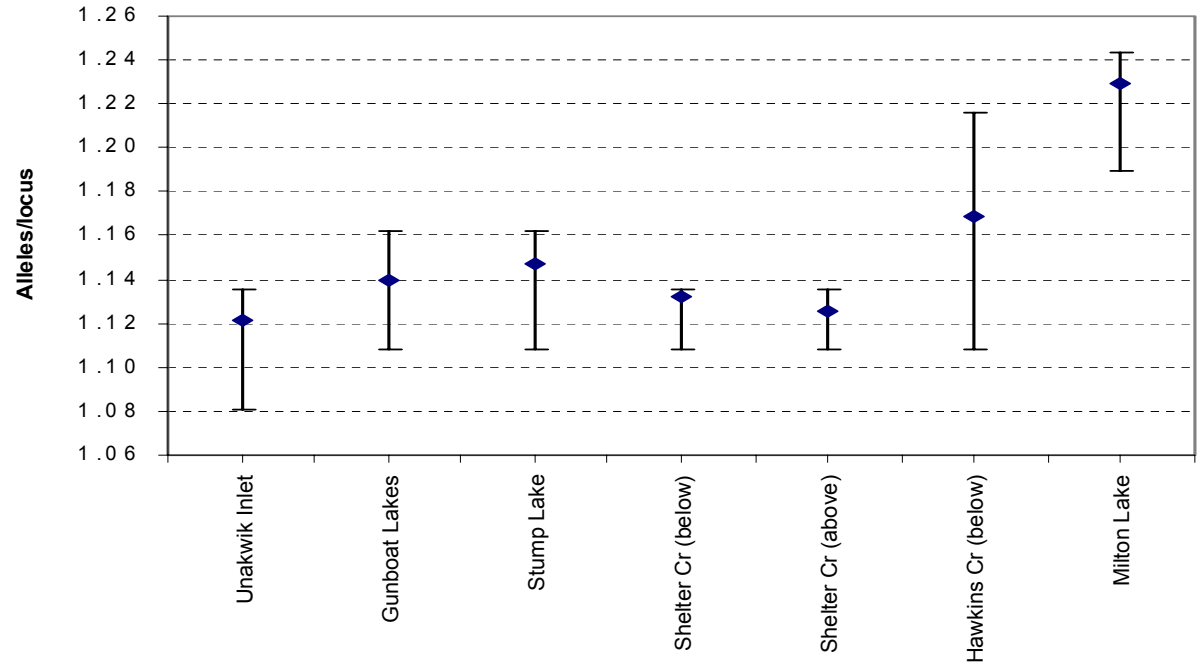
		Collection Location												
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13
Sfo8	237	0.000	0.000	0.000	0.115	0.000	0.000	0.032	0.000	0.026	0.015	0.000	0.000	0.000
	239	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
	243	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ssa85	N	17	27	30	13	25	10	17	30	19	26	21	27	16
	98	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	102	0.000	0.074	0.017	0.000	0.000	0.000	0.059	0.033	0.000	0.019	0.000	0.000	0.031
	108	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	122	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
	124	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	126	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
	128	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	130	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	134	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.024	0.019	0.031
	136	0.000	0.000	0.000	0.000	0.020	0.000	0.088	0.000	0.053	0.077	0.214	0.037	0.031
	138	0.147	0.000	0.050	0.000	0.000	0.000	0.059	0.000	0.026	0.019	0.024	0.019	0.063
	140	0.000	0.037	0.283	0.000	0.060	0.100	0.029	0.050	0.105	0.154	0.000	0.111	0.000
	142	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
	144	0.235	0.426	0.083	0.077	0.080	0.000	0.088	0.017	0.053	0.000	0.000	0.130	0.094
	146	0.412	0.093	0.167	0.500	0.000	0.000	0.206	0.300	0.211	0.154	0.095	0.056	0.000
	148	0.000	0.000	0.133	0.000	0.000	0.000	0.059	0.033	0.053	0.038	0.071	0.000	0.125
	150	0.000	0.037	0.017	0.038	0.000	0.000	0.029	0.100	0.026	0.038	0.095	0.093	0.094
	152	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.026	0.077	0.143	0.000	0.031
	154	0.000	0.000	0.000	0.000	0.020	0.000	0.029	0.017	0.000	0.000	0.071	0.037	0.000
	156	0.000	0.019	0.000	0.000	0.040	0.000	0.029	0.100	0.053	0.058	0.024	0.130	0.000
	158	0.000	0.222	0.200	0.000	0.180	0.100	0.029	0.000	0.000	0.058	0.071	0.037	0.000

Table 7. Continued.

		Collection Location												
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13
Ssa85	160	0.147	0.000	0.017	0.000	0.120	0.000	0.000	0.000	0.000	0.019	0.024	0.056	0.000
	162	0.000	0.000	0.000	0.000	0.160	0.100	0.000	0.067	0.079	0.000	0.024	0.037	0.125
	164	0.000	0.000	0.000	0.000	0.160	0.100	0.059	0.100	0.105	0.115	0.024	0.056	0.188
	166	0.000	0.019	0.000	0.000	0.000	0.000	0.029	0.000	0.053	0.000	0.000	0.037	0.063
	168	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.017	0.000	0.038	0.000	0.000	0.063
	170	0.000	0.019	0.000	0.000	0.040	0.000	0.059	0.033	0.026	0.038	0.071	0.000	0.000
	176	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	180	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	184	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.038	0.000	0.000	0.063
	186	0.029	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.058	0.024	0.000	0.000
	188	0.029	0.000	0.000	0.154	0.000	0.200	0.059	0.033	0.132	0.000	0.000	0.074	0.000
	190	0.000	0.000	0.000	0.192	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	192	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000
	194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000

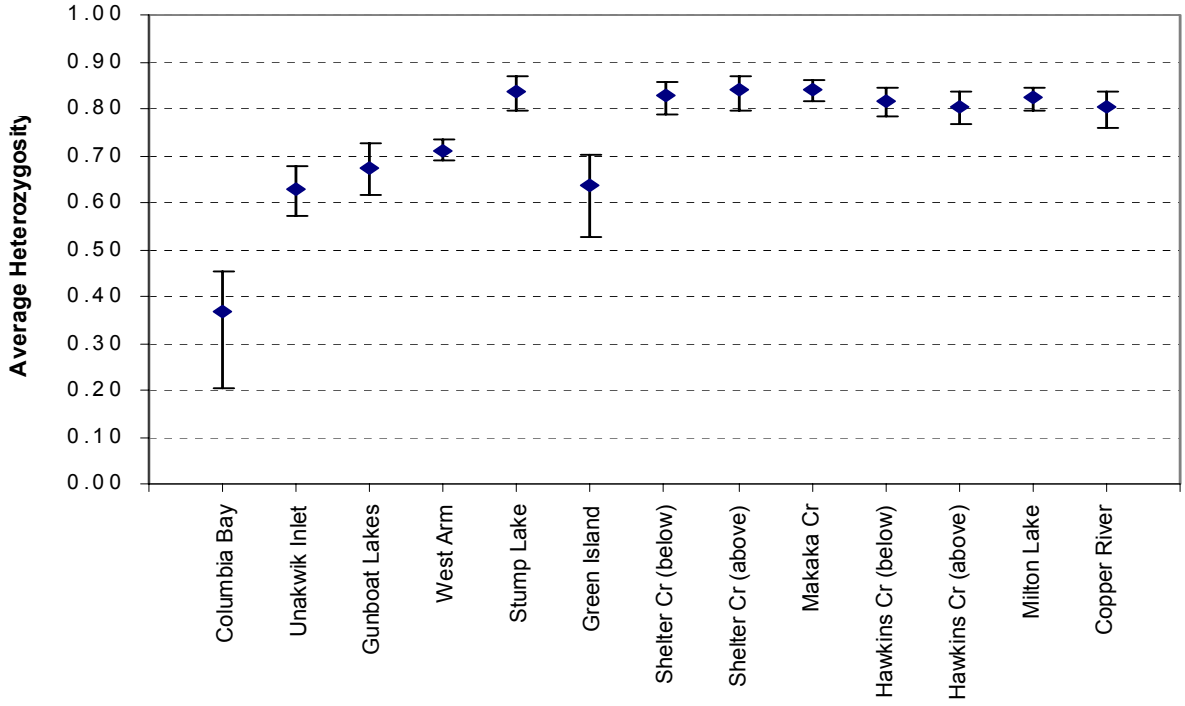


(a)

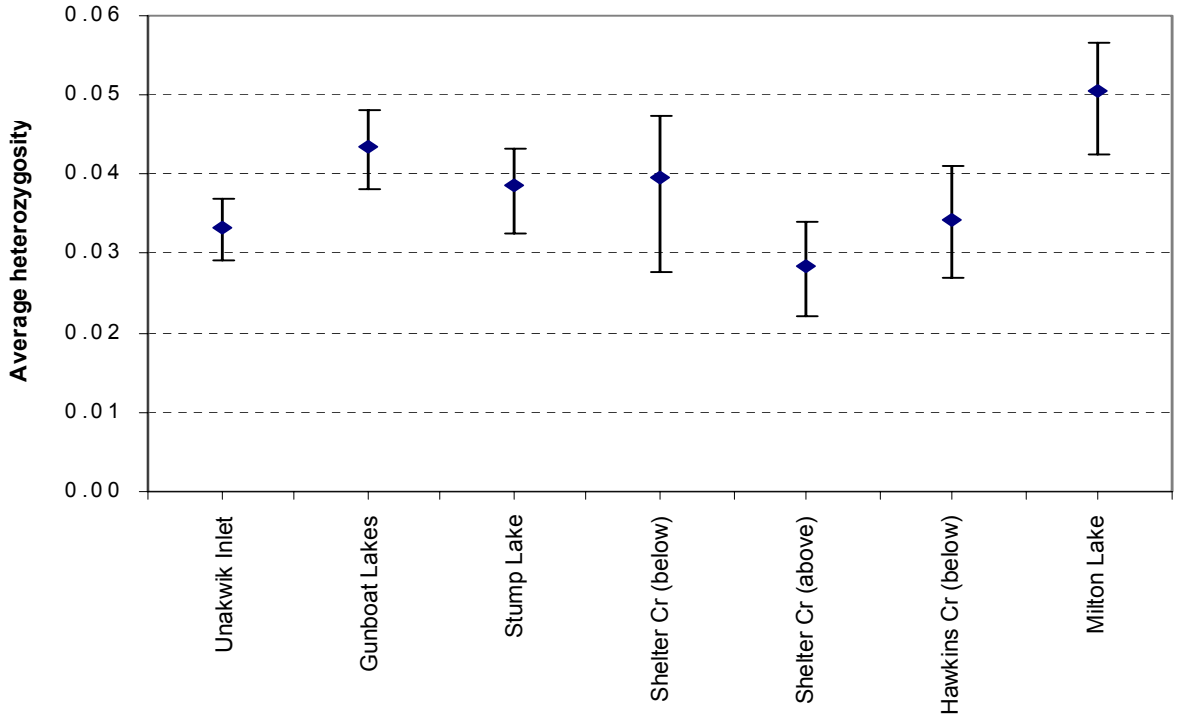


(b)

Figure 3. Mean number of alleles per locus and 95% bootstrap confidence intervals for coastal cutthroat trout in Prince William Sound based on microsatellite DNA (a) and allozyme (b) variation.



(a)



(b)

Figure 4. Nei's unbiased average heterozygosity and 95% bootstrap confidence intervals for coastal cutthroat trout in Prince William Sound based on microsatellite DNA (a) and allozyme (b) variation.

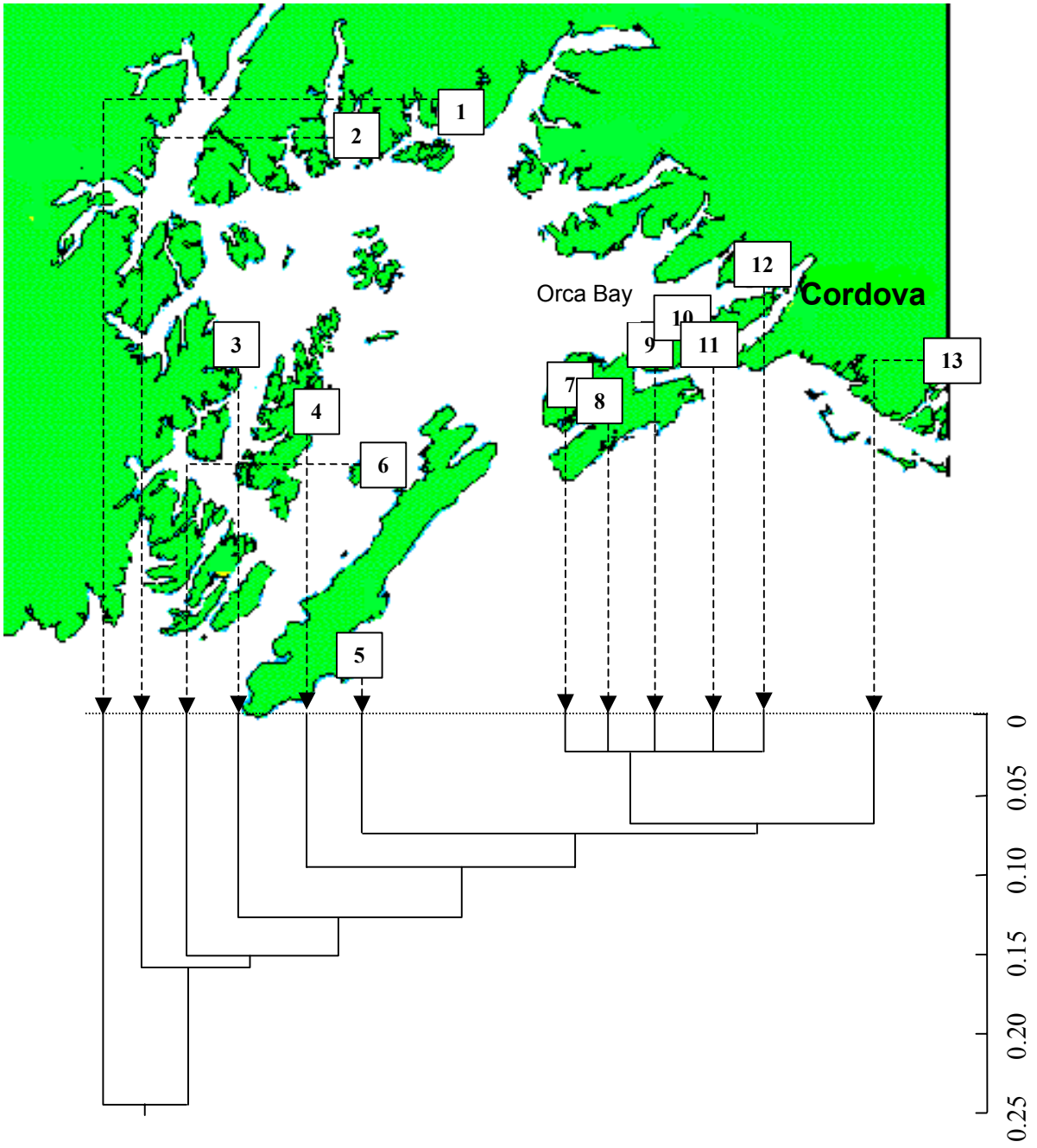


Figure 5. Genetic similarity of PWS coastal cutthroat trout based on coancestry distance.

Table 8. F_{ST} (estimated by θ) and 95% confidence intervals (CI) for Prince William Sound coastal cutthroat trout and Dolly Varden. Asterisks indicate significant genetic differentiation.

	Differentiation Associated with Natal Habitats							
	Island vs Mainland				East vs West			
	Among Regional Groups		Among Local Populations		Among Regional Groups		Among Local Populations	
Analysis	θ_P	95% C.I.	θ_S	95% C.I.	θ_P	95% C.I.	θ_S	95% C.I.
Cutthroat Trout								
mtDNA	-0.007	-	-0.019	-	-0.023	-	-0.025	-
allozyme	-0.007	(-0.037, 0.028)	0.091	(0.053, 0.121)*	-0.028	(-0.040, -0.017)	0.084	(0.033, 0.114)*
msatDNA	0.003	(-0.010, 0.015)	0.112	(0.069, 0.157)*	-0.008	(-0.016, 0.003)	0.107	(0.066, 0.156)*
Dolly Varden (all locations)								
mtDNA	0.104	-	0.448	-	0.069	-	0.401	-
allozyme	-0.015	(-0.026, 0.002)	0.106	(0.053, 0.144)*	0.000	(-0.009, 0.007)	0.115	(0.054, 0.157)*
msatDNA	0.003	(-0.002, 0.009)	0.077	(0.050, 0.102)*	-0.003	(-0.004, -0.004)	0.073	(0.043, 0.102)*
combined	-0.009	(-0.021, 0.003)	0.096	(0.060, 0.131)*	-0.001	(-0.007, 0.005)	0.101	(0.059, 0.142)*
Dolly Varden (unisolated)								
mtDNA	-0.145	-	0.439	-	-0.052	-	0.353	-
allozyme	0.003	(-0.005, 0.012)	0.043	(0.028, 0.057)*	0.005	(-0.004, 0.015)	0.044	(0.029, 0.057)*
msatDNA	0.007	(0.005, 0.009)*	0.041	(0.036, 0.046)*	-0.006	(-0.007, -0.004)	0.034	(0.031, 0.038)*
combined	0.004	(-0.001, 0.011)	0.042	(0.032, 0.053)*	0.002	(-0.005, 0.011)	0.040	(0.030, 0.052)*

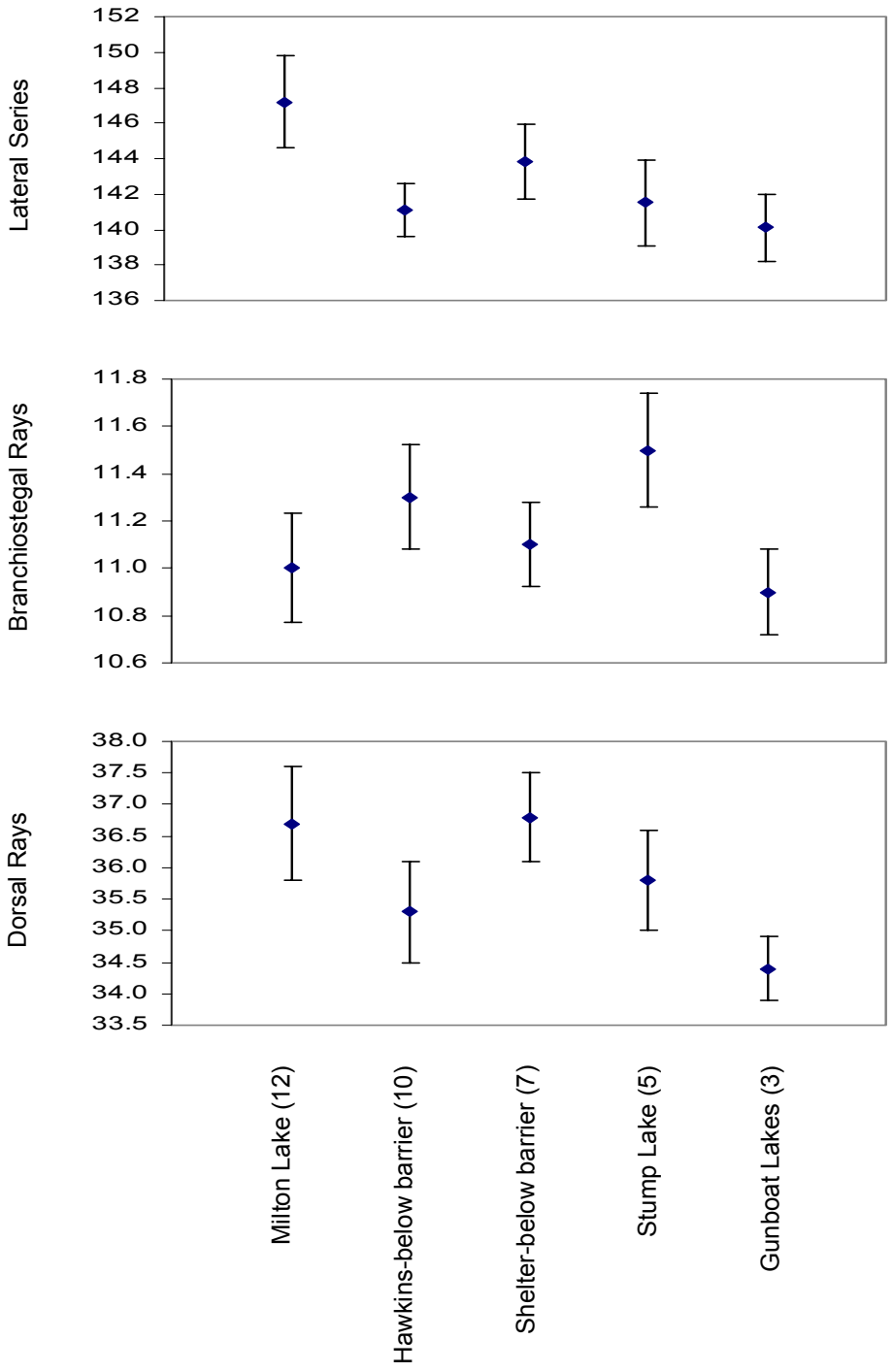


Figure 6. Mean and 95% LSD for counts of the lateral series, branchiostegal rays, and dorsal fin rays for coastal cutthroat trout in Prince William Sound. Numbers in parentheses refer to locations in Figure 1.

locations to the west around the outer edge of PWS (Figure 5). We also found no evidence that geographical genetic structure was associated with differences in natal spawning habitats. No differences were detected between eastern and western aggregations, which generally have different spawning substrates (Table 8). Likewise, we found no differences between cutthroat trout from the Prince William Sound Islands ecosection and mainland ecosections (Table 8).

Although no regional geographical differences were apparent, tests for genetic differentiation based on both allozyme and microsatellite DNA variation did show significant differences among local aggregations (Table 8). No significant differences were detected between samples collected in different years from the same location. Estimates of θ_S for allozyme variation were 0.091 and 0.084 for east versus west and island versus mainland hypotheses, respectively. Microsatellite DNA differentiation was similar to allozyme differentiation, with θ_S of 0.112 and 0.107, respectively.

We also detected significant differences in meristic variation among coastal cutthroat trout in lateral series counts, branchiostegal rays, and dorsal rays (Figure 6). No geographical pattern existed in meristic variation, however, except for a tendency for cutthroat trout from the Gunboat Lakes in the western PWS to have fewer segmented counts than cutthroat trout from other more easterly locations.

We hypothesized that isolation by distance could represent an alternative mode of geographical genetic structure. This hypothesis was supported by the microsatellite DNA cluster analysis that showed increasing stepwise genetic divergence of more geographically more distant aggregations of cutthroat trout from the geographically and genetically similar aggregations around Orca Bay (Figure 5). Mantel tests for significant correlation between pairwise coancestry genetic distances (θ) and geographical genetic distances also supported this hypothesis. We detected significant correlation between pairwise coancestry genetic distances and geographical distances when geographical distances were measured stepwise along the possible post-glacial colonization route from east to west around the circumference of PWS ($p = 0.009$) or according to the shortest possible shoreline distance ($p = 0.015$). We found no significant correlation with shortest possible geographical distances, which were measured assuming that coastal cutthroat trout could migrate across open waters of PWS ($p = 0.128$).

Pairwise estimates of migrants per generation, $\hat{M}$, ranged from 0.59 to 208. In general, gene flow and genetic drift were approximately at equilibrium among coastal cutthroat trout in Prince William Sound. Genetic exchange decreased with increasing geographical distance among aggregations (Figure 7). The slopes of the isolation by distance regressions, assuming east to west dispersal around the circumference of PWS or the shortest shoreline distances, were –0.836 and –0.882. Overall, isolation by distance relationships were the same over all spatial scales ($F = 0.094$, $p = 0.76$), but considerable variability existed in slopes of the isolation by distance relationships at shorter (< 5-70 km) and moderate (100-150 km) distances between aggregations (Figure 8). At shorter distances, which largely included aggregations around Orca Bay (Figure 1), isolation by distance relationships varied widely between slopes of –0.18 to –2.27, suggesting lack of any significant trend in differentiation. Genetic exchange among aggregations around Orca Bay was high, ranging from 15 to 208 migrants per generation. In addition, some of the

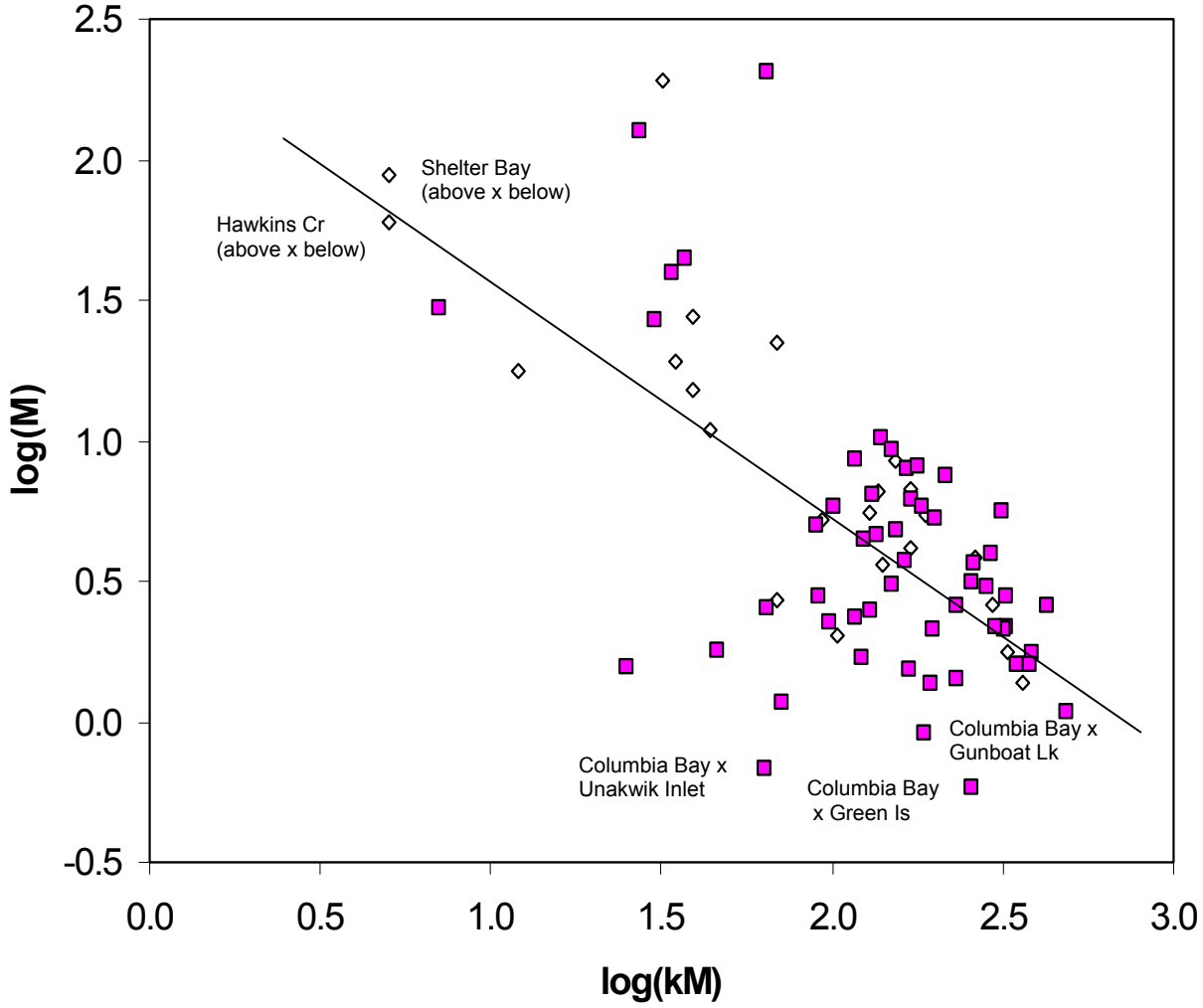


Figure 7. Relationship between gene flow ($\hat{M}$) and geographical distance for Prince William Sound coastal cutthroat trout. Diamonds and squares are populations above and below putative barriers, respectively. Regression line: log ($\hat{M}$) = -0.836x + 2.43 with R^2 value of 0.44.

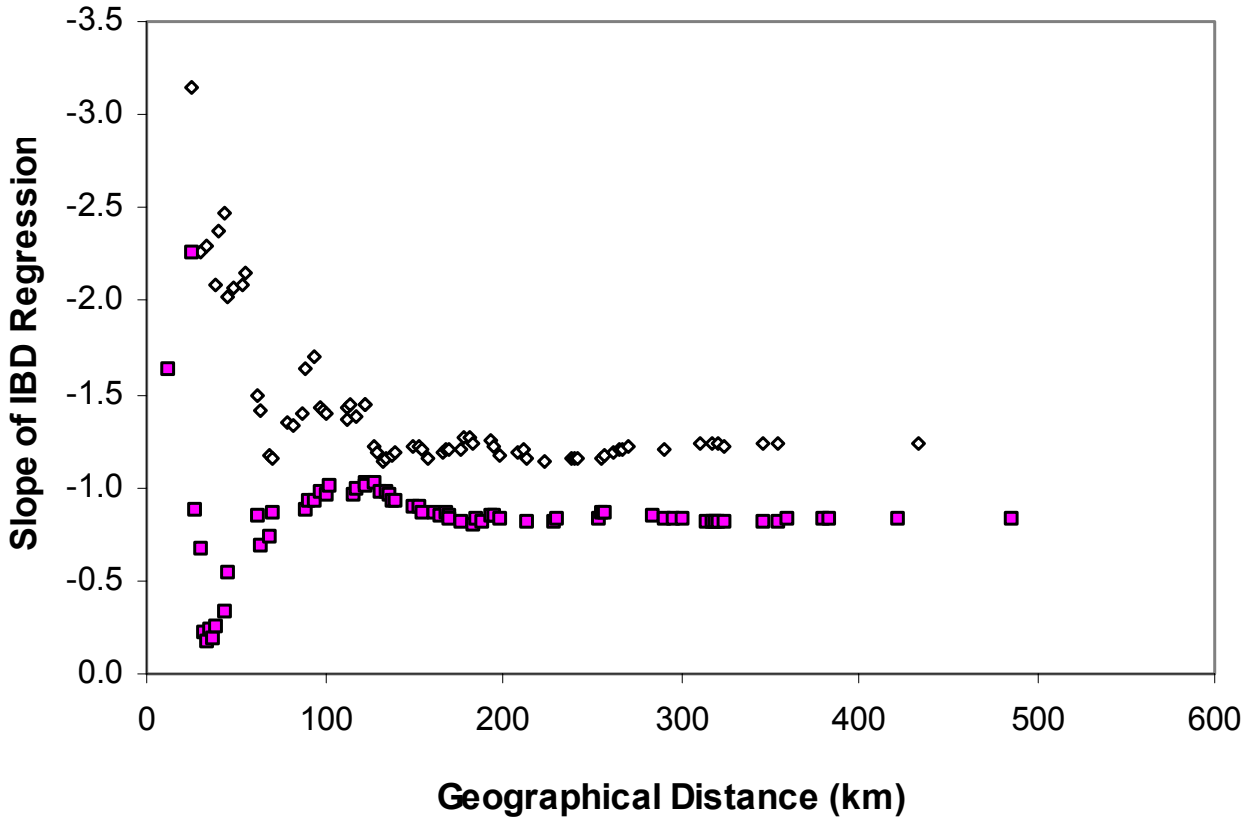


Figure 8. Changes in slope of isolation by distance (IBD) regressions for coastal cutthroat trout (open diamonds) and Dolly Varden charr (closed squares) at different spatial scales in Prince William Sound.

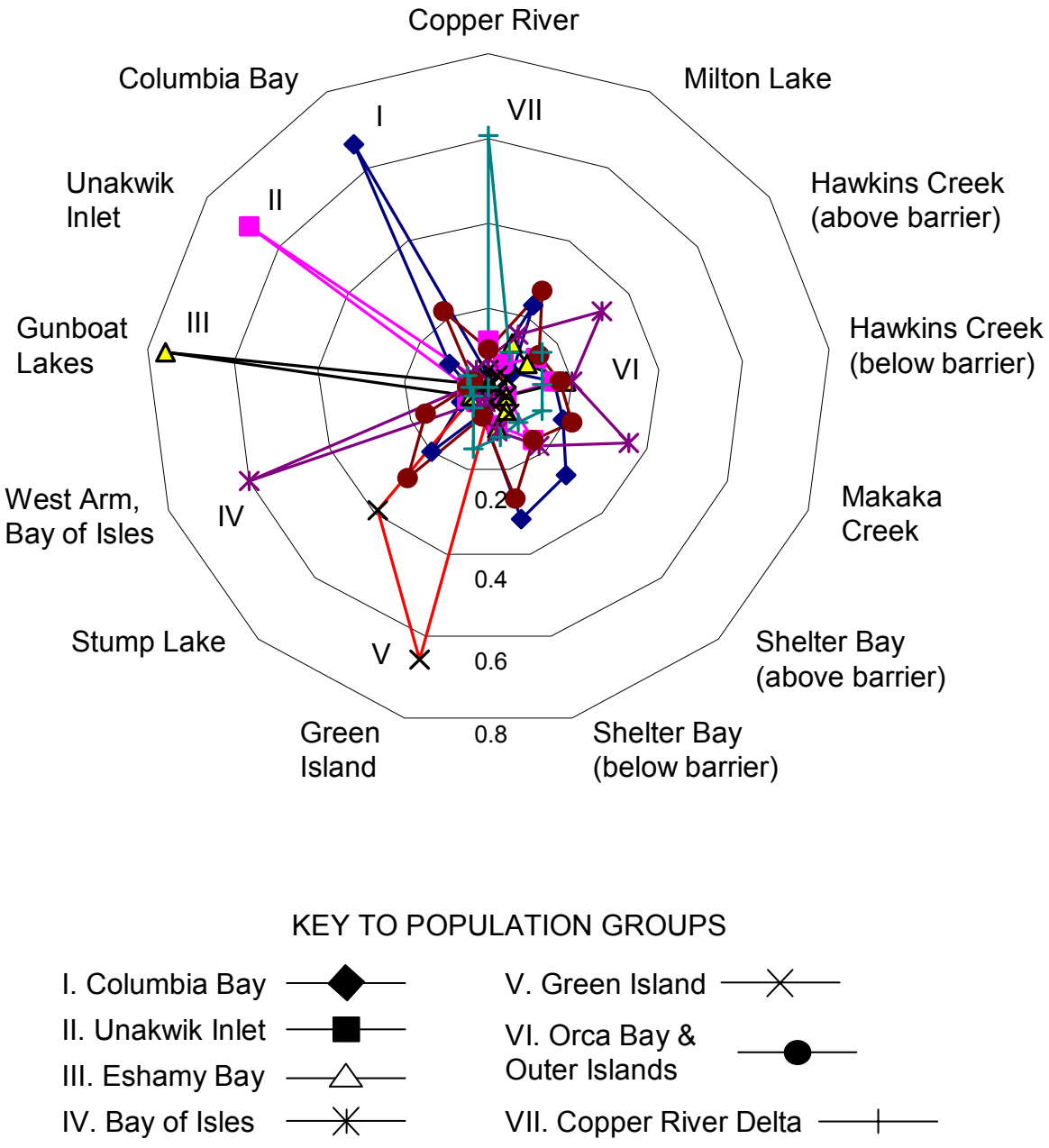


Figure 9. Hypothesized population groups identified by Bayesian cluster analysis assuming an island model of migration and resolving Hardy-Weinberg and linkage disequilibrium in coastal cutthroat trout microsatellite DNA variation. Symbols and Roman numerals indicate hypothesized population groups. Direction and distance towards the 13 different sampling locations in Prince William Sound (around the circumference) indicate the genetic proportions in the samples.

highest rates of genetic exchange occurred between aggregations above and below putative barriers in Shelter Bay and Hawkins Creek (Figure 7). At moderate distances of isolation, slopes peaked near –1.0 and declined monotonously to the mean value, suggesting greater isolation at that geographical scale (Figure 8). These distances largely reflect the geographical proximity of aggregations in the northwestern region of Prince William Sound, which had the lowest genetic exchange. All values of $\hat{M} < 1$ occurred between aggregations in Columbia Bay, Unakwik Inlet, Gunboat Lakes, and Green Island (Figure 8).

We hypothesized that vicariance would have resulted in allopatric differentiation of populations isolated above upstream barriers to migration. We detected no significant differences between cutthroat trout above and below putative barriers in Shelter and Hawkins creeks, however. Likewise mean Sr/Ca ratios in the primordial region of otoliths from cutthroat below barriers in Shelter Creek (0.00298 ± 0.00014) and above barriers (0.00219 ± 0.00096) were not significantly different.

We detected significant deviations from Hardy-Weinberg equilibrium in many aggregations of coastal cutthroat trout. Deviations were due to deficiency of heterozygotes. We detected deviations in allozyme loci at *sMEP-1**, *GDA-2**, and *CK-C2** but in microsatellite loci deficiencies occurred over all loci. Kendall's concordance test indicated that rank differences existed among loci ($W = 0.38$, $p = 0.001$), which might mean that non-amplifying alleles (Callen et al. 1983) or small allele dominance (Wattier et al. 1998) were contributing to deficits. Because of the consistent pattern of heterozygote deficiencies over allozyme and microsatellite loci and the strong possibility that inadvertent sampling of different populations occurred when amphidromous coastal cutthroat trout entered non-natal streams to forage or overwinter, however, we also suspected a Walhund effect. To examine population structure under this scenario, we compared the results of a Bayesian clustering approach (STRUCTURE version 2) that accounts for the presence of Hardy-Weinberg and linkage disequilibrium in the samples with previous results. This analysis, which assumed an island model of migration, showed the same general pattern of genetic differentiation as previous analyses. We identified seven genetically distinct aggregations. These were the aggregations from (1) Columbia Bay, (2) Unakwik Inlet, (3) Gunboat Lakes, (4) West Arm, (5) Stump Lake and Green Island, (6) Orca Bay aggregations (Shelter Bay, Makaka Creek, Hawkins Creek, and Milton Lake), and Copper River (Figure 9). As in other analyses, the most isolated and distinct aggregations, as estimated by the proportion of genetic contribution, were the aggregations from Columbia Bay, Unakwik Inlet, Gunboat Lakes, and West Arm in the northwestern region of PWS. Likewise, the most similar aggregations were the six collections from around Orca Bay. In contrast, whereas genotypic frequencies between Stump Lake and Green Island were significantly different ($P = 0.00001$) and the two exchanged relatively few migrants per generation ($\hat{M} = 2.3$), the STRUCTURE analysis indicated significant migration between these two neighboring islands.

Dolly Varden

We hypothesized that patterns of genetic diversity in Dolly Varden, which are at the center of their range in Prince William Sound, would be different from coastal cutthroat trout. We successfully examined genotypic variation for mtDNA, 50 allozyme loci, and two microsatellite

Table 9. Mitochondrial DNA haplotype frequencies in PWS Dolly Varden. Letters of haplotypes refer to restriction fragment-size patterns when ND-1 was restricted with BstU I, Hae III, and Msp I. Numbers refer to locations in Figure 2. Number of nucleotide substitutions, $\hat{d}$, between AAA, BBB, ACA, and ABB was 0.037, 0.026, and 0.0154, respectively; between BBB, ACA, and ABB was 0.007 and 0.025; and between ACA and ABB was 0.0154.

	Location	N	Haplotype			
			AAA	BBB	ACA	ABB
(1)	Unakwik Inlet	8	1.000	-	-	-
(3)	Eshamy Bay	6	1.000	-	-	-
(4)	West Arm	7	0.143	0.857	-	-
(5)	Hanning Bay	10	0.500	0.500	-	-
(9)	Shelter Creek (below)	9	1.000	-	-	-
(11)	Hawkins Creek (below)	10	0.600	0.100	0.200	0.100
(12)	Hawkins Creek (above)	15	1.000	-	-	-
(14)	Power Creek (below)	11	0.875	0.125	-	-
(15)	Power Creek (above)	7	0.857	0.143	-	-
(16)	Clear Creek	7	1.000	-	-	-

Table 10. Allozyme frequencies and sample sizes (N) for PWS Dolly Varden. Collection site numbers refer to locations in Figure 2.

		Collection Location													
Locus	Allele	16	3	7	5	12	11	4	13	15	14	8	10	9	2
6	1														
*sAAT-2**															
N	43	25	12	71	41	54	62	39	20	41	19	68	23	19	83	37
100	1.000	0.980	0.958	1.000	0.951	1.000	0.984	1.000	1.000	1.000	1.000	0.993	1.000	1.000	1.000	1.000
75	0.000	0.020	0.042	0.000	0.049	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
115	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
*sAAT-4**															
N	42	24	12	67	44	54	65	39	23	42	19	67	21	19	80	34
100	0.786	0.958	0.875	0.948	0.989	0.917	1.000	0.910	0.609	0.798	0.895	0.888	0.952	1.000	0.938	0.956
13	0.214	0.042	0.125	0.052	0.011	0.074	0.000	0.090	0.391	0.202	0.105	0.097	0.048	0.000	0.063	0.044
138	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000
*sAH**															
N	43	24	11	70	43	52	65	40	23	42	19	67	20	19	82	37
100	0.256	0.417	0.545	0.464	0.000	0.510	0.831	0.500	0.217	0.357	0.447	0.440	0.500	0.316	0.378	0.635
94	0.384	0.542	0.364	0.471	0.000	0.346	0.123	0.400	0.783	0.607	0.421	0.485	0.450	0.474	0.537	0.324
86	0.360	0.042	0.091	0.064	1.000	0.144	0.046	0.100	0.000	0.036	0.132	0.075	0.050	0.211	0.085	0.041
*mAH-1**															
N	43	26	12	67	44	54	66	40	22	43	19	69	28	14	82	37
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000	1.000	1.000	1.000	0.994	1.000
63	0.000	0.000	0.000	00.00	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000

Table 10. Continued.

Locus	Allele	Collection Location 16	3	7	5	12	11	4	13	15	14	8	10	9	2	6	1
*mAH-2**																	
	N	43	23	12	64	44	54	65	40	22	43	19	66	27	8	82	34
	100	1.000	0.913	1.000	0.992	1.000	1.000	0.992	0.975	1.000	0.942	1.000	1.000	1.000	0.938	1.000	0.912
	88	0.000	0.087	0.000	0.008	0.000	0.000	0.008	0.025	0.000	0.058	0.000	0.000	0.000	0.063	0.000	0.088
*FDHG**																	
	N	43	29	13	72	46	55	66	40	23	43	19	71	29	20	83	37
	100	1.000	1.000	0.962	0.993	1.000	0.991	0.992	1.000	1.000	1.000	1.000	0.965	0.914	0.925	0.976	1.000
	127	0.000	0.000	0.038	0.007	0.000	0.009	0.008	0.000	0.000	0.000	0.000	0.035	0.086	0.000	0.024	0.000
	150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.000	0.000
*bGLUA**																	
	N	42	23	12	71	44	52	62	39	17	42	19	65	28	17	78	37
	100	0.893	0.957	0.958	0.944	0.966	0.933	0.895	0.923	1.000	0.940	1.000	0.908	0.911	1.000	0.936	0.946
	114	0.048	0.000	0.042	0.035	0.000	0.019	0.056	0.013	0.000	0.012	0.000	0.054	0.071	0.000	0.013	0.027
	78	0.024	0.043	0.000	0.014	0.034	0.048	0.048	0.064	0.000	0.036	0.000	0.038	0.018	0.000	0.038	0.014
	90	0.036	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.013	0.014
*GPIB-1**																	
	N	42	28	13	71	45	53	66	40	1	43	19	49	29	20	80	37
	100	1.000	0.982	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	36	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*GPIB-2**																	
	N	42	27	13	72	46	54	66	40	22	43	19	71	29	20	83	37
	100	1.000	1.000	0.962	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	133	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 10. Continued.

		Collection Location															
Locus	Allele	16	3	7	5	12	11	4	13	15	14	8	10	9	2	6	1
*GPI-A**																	
	N	43	29	12	72	45	46	66	40	23	43	19	66	29	20	76	37
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000	0.925	0.993	1.000
	88	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
	110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.007	0.000
*G3PHD-2**																	
	N	43	29	12	72	44	54	63	40	22	41	19	60	27	20	83	37
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.984	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.986
	62	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
*IDDH-1**																	
	N	41	25	12	69	40	52	65	40	23	40	19	65	20	19	69	33
	100	1.000	0.980	1.000	1.000	1.000	1.000	0.985	0.988	1.000	1.000	1.000	1.000	1.000	0.974	1.000	1.000
	188	0.000	0.020	0.000	0.000	0.000	0.000	0.015	0.013	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000
*sIDHP-2**																	
	N	43	28	12	72	46	54	66	40	23	43	19	72	29	20	83	36
	100	1.000	0.982	0.917	0.993	1.000	1.000	0.977	0.962	0.935	0.977	1.000	1.000	1.000	0.975	1.000	1.000
	121	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.023	0.000	0.000	0.000	0.000	0.000	0.000
	81	0.000	0.000	0.083	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
	111	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	115	0.000	0.018	0.000	0.000	0.000	0.000	0.008	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*LDHB-2**																	
	N	43	30	12	72	46	54	66	40	23	43	19	71	29	20	82	37
	100	1.000	1.000	1.000	0.993	1.000	0.991	1.000	0.988	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000
	62	0.000	0.000	0.000	0.007	0.000	0.009	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000

Table 10. Continued.

		Collection Location															
Locus	Allele	16	3	7	5	12	11	4	13	15	14	8	10	9	2	6	1
*LDHC**																	
	N	41	20	12	70	46	52	64	39	23	41	16	69	27	20	82	33
	100	0.561	0.675	0.792	0.721	1.000	0.635	0.766	0.628	0.457	0.573	0.875	0.601	0.630	0.975	0.646	0.803
	97	0.390	0.200	0.167	0.214	0.000	0.288	0.195	0.333	0.000	0.195	0.125	0.348	0.333	0.025	0.329	0.197
	94	0.049	0.125	0.042	0.064	0.000	0.077	0.039	0.038	0.543	0.232	0.000	0.051	0.037	0.000	0.024	0.000
*SMDHA-2**																	
	N	43	26	12	72	45	54	66	40	23	43	19	72	29	20	83	37
	100	0.895	0.923	0.958	0.882	1.000	0.935	0.939	0.887	1.000	0.977	0.868	0.861	0.845	0.850	0.934	1.000
	70	0.105	0.077	0.042	0.118	0.000	0.065	0.061	0.112	0.000	0.023	0.132	0.139	0.155	0.150	0.066	0.000
*sMDHB-2**																	
	N	43	26	12	72	45	53	66	40	23	43	19	69	28	20	83	37
	100	0.965	1.000	1.000	0.993	1.000	0.943	1.000	1.000	1.000	1.000	1.000	0.949	0.982	0.950	1.000	0.973
	75	0.000	0.000	0.000	0.007	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.051	0.018	0.000	0.000	0.000
	115	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027
	67	0.035	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000
	133	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*MPI**																	
	N	43	23	13	70	43	52	65	39	22	42	19	68	26	20	82	37
	100	0.907	0.978	1.000	0.986	1.000	0.990	0.992	1.000	1.000	0.988	0.974	0.978	1.000	0.900	0.982	1.000
	87	0.000	0.022	0.000	0.014	0.000	0.010	0.008	0.000	0.000	0.012	0.026	0.022	0.000	0.000	0.018	0.000
	104	0.093	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	96	0.000	0.000	.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000

Table 10. Continued.

Locus	Allele	Collection Location 16	3	7	5	12	11	4	13	15	14	8	10	9	2	6	1
*NTP**																	
	N	43	28	12	72	36	51	66	40	19	36	19	65	22	19	83	37
	100	0.616	0.911	0.833	0.840	0.875	0.716	0.773	0.762	0.974	0.903	0.842	0.623	0.614	0.816	0.843	0.689
	450	0.384	0.089	0.167	0.160	0.125	0.284	0.227	0.237	0.026	0.097	0.158	0.377	0.386	0.184	0.157	0.311
*PEP-B1**																	
	N	43	29	12	72	46	54	66	40	23	43	19	72	29	20	83	37
	100	0.826	0.793	0.917	0.882	0.174	0.778	0.811	0.837	0.652	0.721	0.921	0.903	0.862	0.800	0.873	0.568
	74	0.174	0.207	0.083	0.118	0.826	0.222	0.189	0.162	0.348	0.279	0.079	0.097	0.138	0.200	0.127	0.432
*PEP-D1**																	
	N	42	25	12	71	44	51	64	40	23	34	19	68	29	19	81	35
	100	0.940	0.960	0.792	0.824	1.000	0.784	0.898	0.913	1.000	0.868	0.842	0.890	0.914	1.000	0.827	0.671
	119	0.048	0.040	0.208	0.176	0.000	0.216	0.102	0.087	0.000	0.132	0.158	0.110	0.086	0.000	0.173	0.329
	80	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*PEP-D2**																	
	N	38	22	12	69	38	48	62	39	5	27	17	50	24	18	72	16
	100	0.934	1.000	1.000	1.000	1.000	0.969	0.992	0.987	1.000	1.000	1.000	1.000	1.000	1.000	0.951	1.000
	112	0.066	0.000	0.000	0.000	0.000	0.031	0.008	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.049	0.000
*PEP-LT**																	
	N	41	25	12	70	45	50	64	38	23	42	19	67	29	18	79	36
	100	0.890	0.940	1.000	0.893	1.000	0.900	0.938	0.908	1.000	0.940	0.895	0.903	0.966	1.000	0.854	0.736
	86	0.000	0.060	0.000	0.079	0.000	0.020	0.047	0.053	0.000	0.060	0.053	0.067	0.017	0.000	0.025	0.028
	66	0.061	0.000	0.000	0.029	0.000	0.050	0.008	0.039	0.000	0.000	0.026	0.030	0.017	0.000	0.025	0.236
	53	0.049	0.000	0.000	0.000	0.000	0.030	0.008	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.095	0.000

Table 10. Continued.

		Collection Location															
Locus	Allele	16	3	7	5	12	11	4	13	15	14	8	10	9	2	6	1
*PGDH**																	
	N	42	28	12	72	46	54	66	40	23	43	19	72	29	20	83	37
	100	0.786	0.964	1.000	1.000	1.000	0.981	0.992	0.975	1.000	1.000	1.000	0.979	1.000	1.000	0.994	0.959
	83	0.214	0.036	0.000	0.000	0.000	0.019	0.008	0.025	0.000	0.000	0.000	0.021	0.000	0.000	0.006	0.041
*PGM-1**																	
	N	43	29	13	71	30	52	66	40	23	43	19	62	29	20	82	37
	100	0.779	0.931	1.000	0.972	1.000	0.923	0.992	0.938	1.000	1.000	0.921	0.960	0.914	0.825	0.982	1.000
	25	0.198	0.034	0.000	0.014	0.000	0.019	0.008	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
	180	0.000	0.034	0.000	0.000	0.000	0.029	0.000	0.025	0.000	0.000	0.053	0.008	0.034	0.125	0.000	0.000
	211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.052	0.000	0.012	0.000
	5	0.012	0.000	0.000	0.014	0.000	0.029	0.000	0.000	0.000	0.000	0.026	0.008	0.000	0.025	0.000	0.000
	160	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.008	0.000	0.025	0.000	0.000
*PGM-2**																	
	N	43	28	13	72	46	54	66	40	23	43	19	71	29	20	83	37
	100	0.942	1.000	1.000	.972	1.000	0.981	1.000	0.975	0.978	1.000	0.974	0.993	1.000	0.975	0.958	1.000
	122	0.058	0.000	0.000	.028	0.000	0.019	0.000	0.025	0.000	0.000	0.026	0.007	0.000	0.025	0.042	0.000
	62	0.000	0.000	0.000	.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*S0D-1**																	
	N	43	27	12	72	46	54	66	40	23	43	19	69	29	20	82	37
	100	0.837	0.685	0.750	0.757	0.000	0.769	0.621	0.725	1.000	0.756	0.711	0.746	0.776	0.800	0.848	0.797
	90	0.151	0.241	0.167	0.118	1.000	0.167	0.356	0.250	0.000	0.105	0.184	0.159	0.138	0.150	0.128	0.203
	94	0.012	0.074	0.083	0.125	0.000	0.056	0.023	0.025	0.000	0.140	0.105	0.087	0.086	0.050	0.024	0.000
	69	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000

Table 10. Continued.

		Collection Location															
Locus	Allele	16	3	7	5	12	11	4	13	15	14	8	10	9	2	6	1
*TPI-4**																	
	N	35	19	12	54	44	48	64	35	23	40	16	62	28	19	70	27
	100	1.000	1.000	1.000	0.954	1.000	1.000	0.992	0.943	1.000	1.000	0.969	1.000	1.000	1.000	0.964	1.000
	96	0.000	0.000	0.000	0.046	0.000	0.000	0.008	0.057	0.000	0.000	0.031	0.000	0.000	0.000	0.036	0.000

Table 11. Microsatellite DNA frequencies and sample sizes (N) for PWS Dolly Varden. Collection site numbers refer to locations in Figure 2.

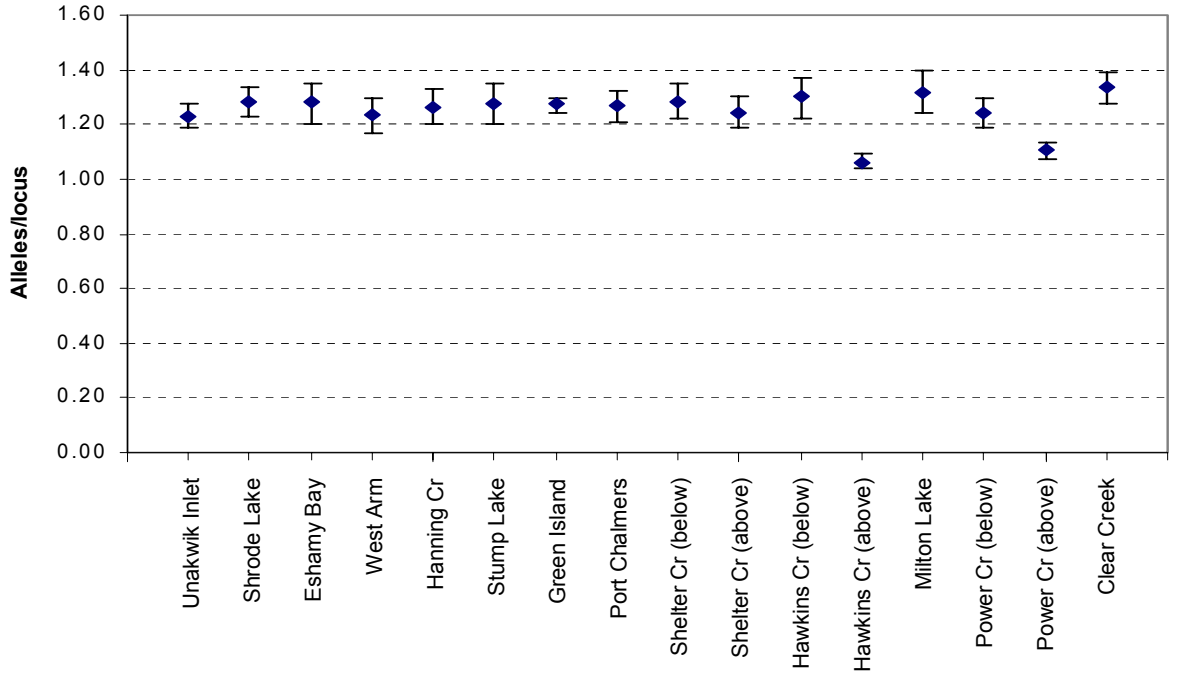
		Collection Location											
Locus	Allele	1	3	4	5	6	7	9	11	12	14	15	16
Sfo8	N	5	6	33	21	23	12	26	14	14	22	11	15
	234	0.200	0.000	0.242	0.167	0.239	0.000	0.173	0.179	0.036	0.205	0.045	0.500
	236	0.100	0.000	0.076	0.214	0.174	0.292	0.038	0.107	0.179	0.205	0.182	0.233
	242	0.600	0.000	0.212	0.071	0.065	0.125	0.115	0.107	0.143	0.159	0.000	0.033
	246	0.000	0.000	0.015	0.048	0.087	0.167	0.154	0.036	0.214	0.091	0.136	0.033
	248	0.000	0.000	0.015	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.100
	250	0.000	0.000	0.000	0.024	0.022	0.000	0.000	0.000	0.000	0.068	0.000	0.033
	252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
	254	0.000	0.000	0.076	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
	256	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	258	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	262	0.000	0.167	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	266	0.000	0.083	0.015	0.000	0.000	0.042	0.019	0.000	0.000	0.000	0.000	0.000
	268	0.000	0.083	0.061	0.048	0.152	0.042	0.096	0.000	0.143	0.000	0.000	0.000
	270	0.000	0.000	0.061	0.119	0.087	0.083	0.096	0.000	0.071	0.000	0.000	0.000
	272	0.000	0.000	0.076	0.095	0.000	0.083	0.019	0.071	0.179	0.000	0.000	0.000
	274	0.100	0.000	0.000	0.048	0.022	0.083	0.058	0.000	0.000	0.000	0.000	0.000
	276	0.000	0.000	0.000	0.000	0.022	0.083	0.019	0.036	0.000	0.000	0.000	0.000
	278	0.000	0.000	0.015	0.024	0.000	0.000	0.019	0.000	0.036	0.068	0.000	0.000
	280	0.000	0.000	0.000	0.024	0.000	0.000	0.019	0.107	0.000	0.091	0.500	0.000
	282	0.000	0.000	0.045	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	288	0.000	0.000	0.015	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000
	292	0.000	0.000	0.000	0.024	0.000	0.000	0.077	0.071	0.000	0.091	0.091	0.000
	294	0.000	0.000	0.030	0.000	0.022	0.000	0.000	0.107	0.000	0.000	0.000	0.000
	296	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.023	0.000	0.000
	298	0.000	0.000	0.000	0.000	0.022	0.000	0.019	0.036	0.000	0.000	0.000	0.000
	300	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.045	0.000

Table 11. Continued.

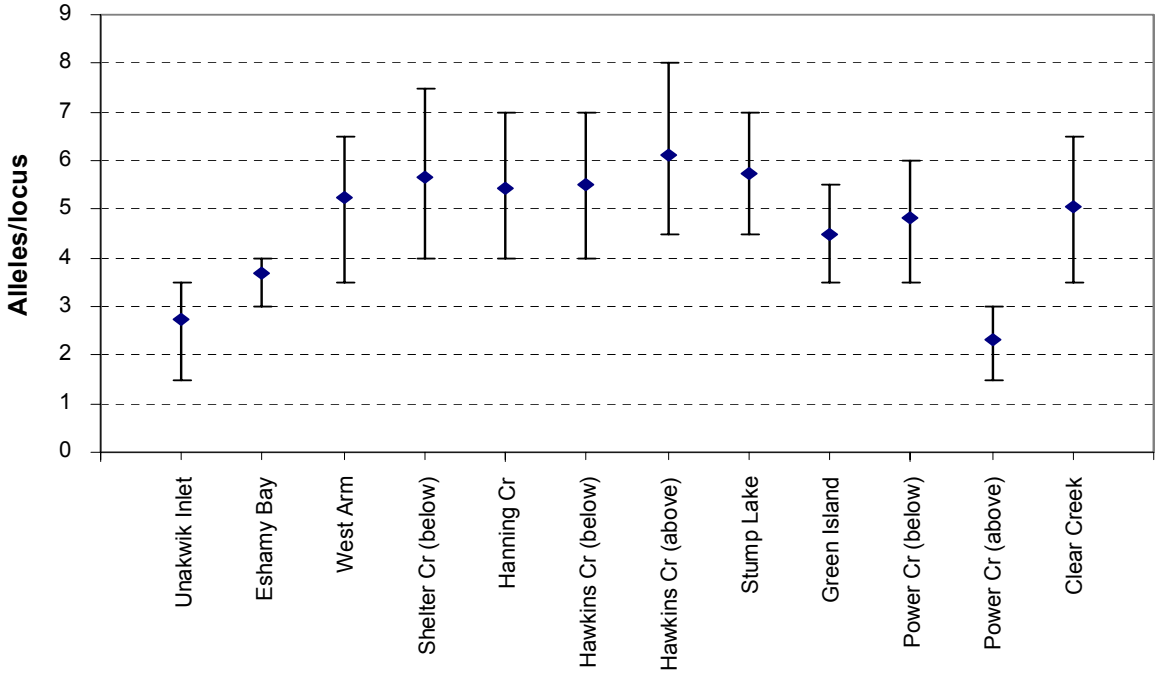
		Collection Location											
Locus	Allele	1	3	4	5	6	7	9	11	12	14	15	16
Sfo8	304	0.000	0.000	0.015	0.000	0.022	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	314	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	318	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ssa85	N	8	6	34	26	25	12	26	20	16	26	13	14
	124	0.438	0.500	0.103	0.077	0.000	0.000	0.096	0.100	0.125	0.038	0.000	0.036
	126	0.125	0.000	0.206	0.019	0.100	0.125	0.115	0.125	0.000	0.077	0.000	0.179
	136	0.000	0.000	0.059	0.077	0.020	0.125	0.000	0.050	0.000	0.058	0.000	0.107
	140	0.000	0.000	0.000	0.135	0.040	0.000	0.000	0.025	0.219	0.038	0.000	0.107
	142	0.000	0.167	0.015	0.019	0.020	0.000	0.077	0.025	0.000	0.000	0.000	0.036
	148	0.000	0.000	0.088	0.000	0.000	0.000	0.038	0.050	0.000	0.000	0.000	0.179
	152	0.000	0.000	0.118	0.038	0.160	0.042	0.077	0.000	0.000	0.115	0.000	0.036
	156	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.036
	158	0.000	0.000	0.147	0.077	0.000	0.000	0.038	0.200	0.000	0.000	0.000	0.071
	164	0.000	0.000	0.000	0.115	0.000	0.042	0.038	0.025	0.281	0.000	0.000	0.071
	166	0.000	0.000	0.118	0.058	0.000	0.208	0.096	0.150	0.250	0.192	0.000	0.107
	168	0.000	0.000	0.015	0.058	0.000	0.000	0.000	0.025	0.031	0.077	0.000	0.036
	172	0.000	0.333	0.029	0.154	0.100	0.000	0.019	0.000	0.000	0.058	0.000	0.000
	174	0.000	0.000	0.000	0.038	0.120	0.125	0.000	0.000	0.000	0.000	0.000	0.000
	176	0.000	0.000	0.000	0.000	0.080	0.083	0.000	0.025	0.000	0.000	0.000	0.000
	178	0.000	0.000	0.000	0.000	0.040	0.042	0.038	0.050	0.000	0.000	0.000	0.000
	182	0.000	0.000	0.029	0.019	0.020	0.042	0.019	0.000	0.000	0.000	0.000	0.000
	186	0.000	0.000	0.029	0.019	0.060	0.167	0.077	0.075	0.000	0.000	0.000	0.000
	188	0.000	0.000	0.000	0.038	0.000	0.000	0.019	0.000	0.094	0.000	0.000	0.000
	192	0.000	0.000	0.000	0.019	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	194	0.000	0.000	0.000	0.019	0.000	0.000	0.019	0.000	0.000	0.038	0.000	0.000
	198	0.000	0.000	0.015	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	200	0.000	0.000	0.000	0.000	0.080	0.000	0.058	0.025	0.000	0.000	0.000	0.000
	202	0.438	0.000	0.000	0.000	0.000	0.000	0.135	0.050	0.000	0.038	0.000	0.000

Table 11. Continued.

		Collection Location											
Locus	Allele	1	3	4	5	6	7	9	11	12	14	15	16
Ssa85	206	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.269	1.000	0.000
	208	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	210	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	214	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	222	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	224	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

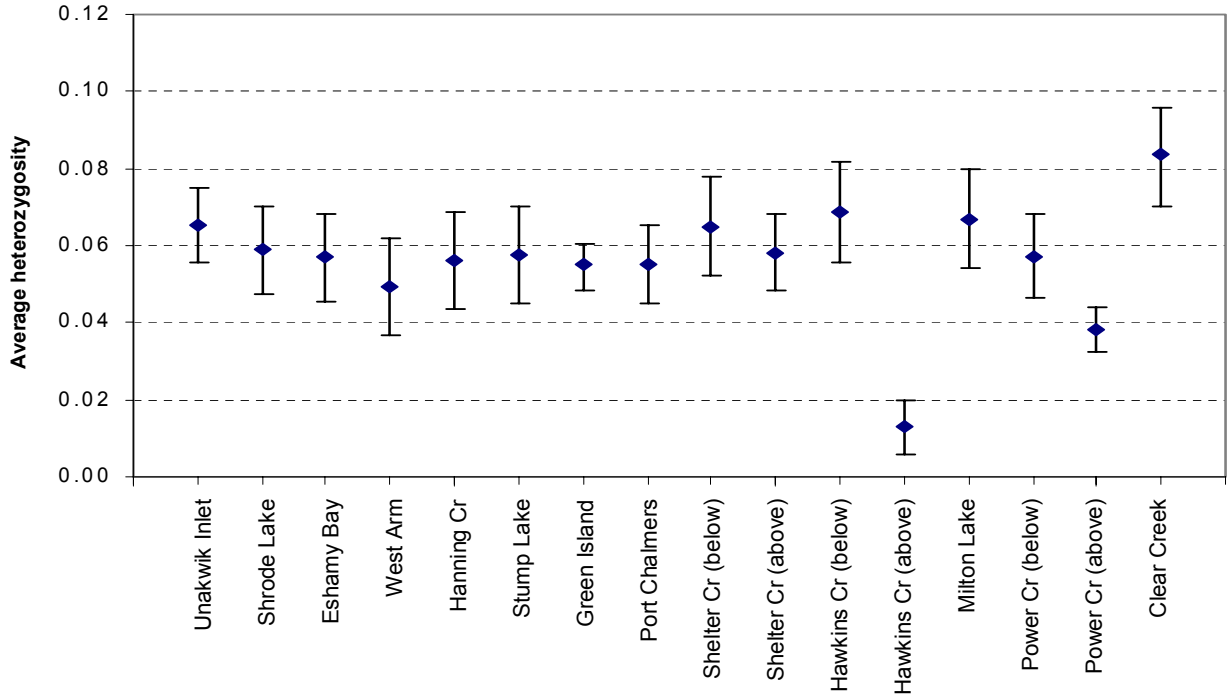


(a)

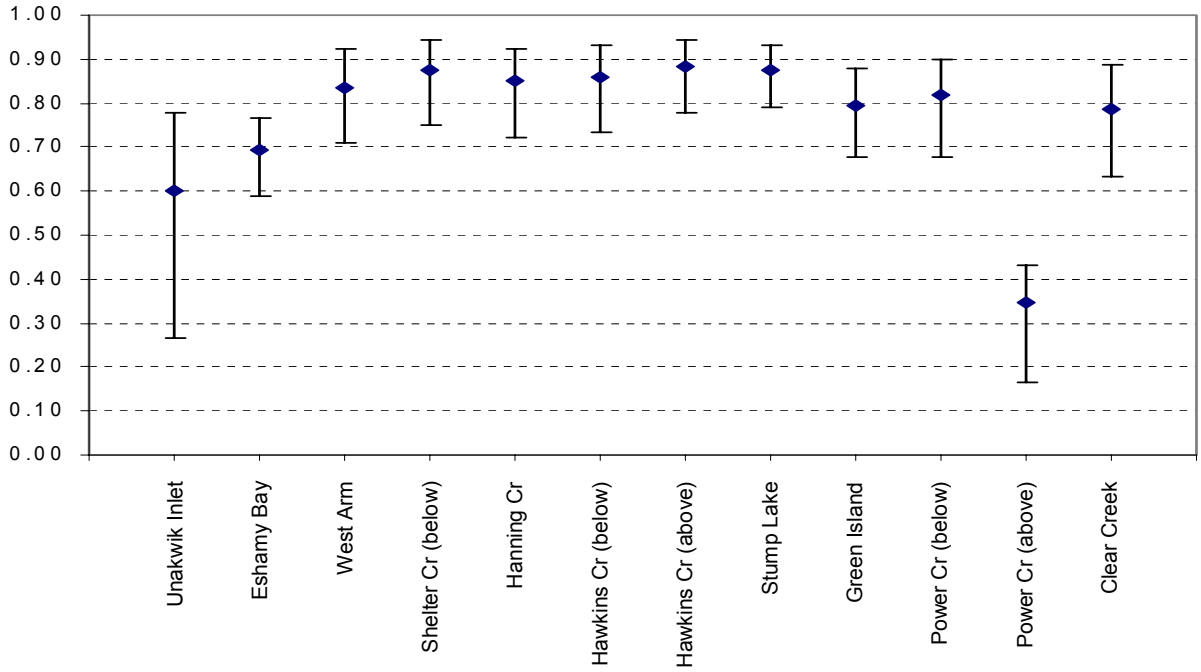


(b)

Figure 10. Mean number of alleles per locus and 95% bootstrap confidence intervals for Dolly Varden in Prince William Sound based on microsatellite DNA (a) and allozyme (b) variation.



(a)



(b)

Figure 11. Nei's unbiased average heterozygosity and 95% bootstrap confidence intervals for Dolly Varden in Prince William Sound based on microsatellite DNA (a) and allozyme (b) variation.

loci in PWS Dolly Varden. Overall, Dolly Varden in Prince William Sound were more polymorphic and had different genetic structure than coastal cutthroat trout.

Four composite mtDNA haplotypes occurred in Dolly Varden (Table 9). Mitochondrial DNA variation was limited to the ND-1 region. Restriction of ND-1 with *BstU I* yielded two visible base-pair (bp) fragment-size patterns: A) 790-, 435-, 260-, 260-, 235-bp; and B) 1020-, 435-, 260-, 235-bp. *Hae III* yielded three visible fragment-size patterns: A) 647-, 380-, 233-, 184-, 172-, 124-, 116-, 82-, 45-bp; B) 647-, 417-, 280-, 184-, 172-, 116-, 88-, 82-bp; and C) 647-, 510-, 280-, 184-, 172-, 116-, 82-bp. *Msp I* yielded two fragment-size patterns: A) 1070-, 470-, 250-, 180-, 125-bp; and B) 660-, 470-, 420-, 250-, 180-, 125-bp.

Dolly Varden were also polymorphic at the 0.99 criterion at 28 of the 50 allozyme loci scored (Table 10). *FH** was not analyzed because of ambiguities in the scoring. Dolly Varden aggregations averaged 1.25 allozyme alleles per locus across the 16 sampling locations (Figure 10) and H_e ranged from 0.013-0.084 (Figure 11). Both of the microsatellite loci that we successfully scored, *Sfo8** and *Ssa85**, were highly polymorphic. Dolly Varden averaged 4.73 alleles per microsatellite DNA locus and H_e ranged from 0.349-0.882. Overall all loci, genotypic proportions conformed to Hardy-Weinberg equilibrium.

Dolly Varden in Prince William Sound had a different pattern of within-aggregation genetic diversity than coastal cutthroat trout. We had hypothesized that populations isolated above barriers would have lower genetic effective sizes and consequently less genetic diversity than those below barriers where gene flow could occur. Although we did not see this in coastal cutthroat trout, in Dolly Varden aggregations isolated above barriers to upstream migration tended to show reduced genetic diversity than those below barriers. In both Power and Hawkins creeks, for example, Dolly Varden above barriers had few microsatellite alleles per locus and lower H_e than those below (Figures 10a, 11a). Allozyme data showed the same pattern for Power Creek but not for Hawkins Creek (Figures 10b, 11b). Over all loci, Dolly Varden in Power Creek above barriers had lower average heterozygosity ($t = -2.004$, $df = 24$, $p = 0.03$), as did those above barriers in Hawkins Creek ($t = -3.853$, $df = 23$, $p = 0.000$), than those below barriers in the same streams. We found no difference in within-population genetic diversity between Dolly Varden above and below barriers in Shelter Creek.

We hypothesized that geographical genetic structure in Dolly Varden in PWS might be associated with different geographical regions. Neither the tests of parapatric differentiation associated with natal habitat nor cluster analyses supported this hypothesis. We found limited evidence that geographical structure was associated with differences in natal spawning habitats. No differences were detected in allozyme or microsatellite DNA variation between eastern and western Dolly Varden aggregations. Likewise, we found no differences between Dolly Varden from different ecosections in PWS (Table 6). Values for θ_P for mtDNA variation, however, suggested possible regional differences. Eastern aggregations had significantly different mtDNA haplotype frequencies ($\theta_P = 0.104$) than western aggregations ($G = 12.88$, $df = 3$, $p = 0.005$). Likewise, island aggregations had significantly different haplotype frequencies ($\theta_P = 0.069$) than mainland aggregations ($G = 11.96$, $df = 3$, $p = 0.008$).

Cluster analysis of mtDNA variation illustrated these differences, but cluster analyses of allozyme and microsatellite DNA variation illustrated a very different pattern of geographical genetic structure. Cluster analyses based on mtDNA variation identified a group of geographically diverse populations characterized by high frequencies of the AAA haplotype (Table 9), and a second group, consisting of Dolly Varden from Hanning Creek and West Arm (both are western and island aggregations) that had high frequencies of the BBB haplotype (Figure 12). In contrast, cluster analyses based on combined allozyme and microsatellite DNA data pointed largely to genetic differences of above- and below-barrier populations (Figure 13). Figure 13 showed a geographically central group of genetically homogenous below-barrier aggregations ($\theta \sim 0$) with minor divergence of geographically peripheral aggregations from Unakwik Inlet, Shrode Lake, Eshamy Bay, West Arm, and Clear Creek ($\theta < 0.05$). The most different populations, however, were the Dolly Varden above barriers in Power and Hawkins creeks.

Although we saw different patterns of regional geographical genetic variation depending on the kind of data, all the analyses showed significant genetic differences among local aggregations of Dolly Varden (Table 8). We had hypothesized that vicariance would have resulted in allopatric differentiation of populations above upstream barriers of migration. In Dolly Varden, this was a major source of genetic divergence. Combined θ_S for allozyme and microsatellite DNA variation, for example, was 0.101 for all populations, but it was only 0.040 when populations above barriers were removed from the analysis (Table 8). Dolly Varden above barriers in Hawkins Creek were significantly different from those below the barrier ($p = 0.0000$) and were fixed for an uncommon allele at *sAH** and *sSOD-1** (Table 10). Dolly Varden in Power Creek were significantly different from those below the barrier ($p = 0.0000$) and were fixed for the *Ssa85*136* allele (Table 11). Dolly Varden above and below barriers in Shelter Creek, however, were not significantly different ($p = 0.969$). Interestingly, estimates of Dolly Varden genetic divergence including above- and below-barrier aggregations was nearly twice that of below - barrier aggregations, although it was comparable to divergence of unisolated coastal cutthroat trout (Table 6).

Although genetic differences existed between Dolly Varden above and below barriers in Power Creek, we found no significant differences in Sr/Ca ratios in the primordial region between the two groups. Mean for anadromous fishes was 0.00096 ± 0.00045 and for resident fishes was 0.00079 ± 0.0001. Below-barrier Dolly Varden, however, had higher Sr/Ca ratios in the outer margin of the otolith, which is consistent with deposition during its marine phase, whereas Sr/Ca ratio above-barrier individuals remained low, consistent with a prolonged freshwater residency (Figure 14).

We hypothesized that isolation by distance could represent an alternative mode of geographical genetic structure in Dolly Varden. Mantel tests for significant correlation between pairwise coancestry genetic distances and geographical genetic distances supported this hypothesis when all below-barrier aggregations were included in the analysis. We detected significant correlation when geographical distances were measured stepwise along the possible post-glacial dispersal route from east to west around the circumference of PWS ($p = 0.002$), along the shortest possible shoreline distance ($p = 0.002$), and by the shortest possible geographical distance ($p = 0.006$), which assumed that Dolly Varden could migrate across open waters of PWS. When genetically

undifferentiated aggregations (Hanning Creek, Green Island, Port Chalmers, and Shelter Bay below barrier) were removed from the analyses, however, we detected no isolation by distance in Dolly Varden.

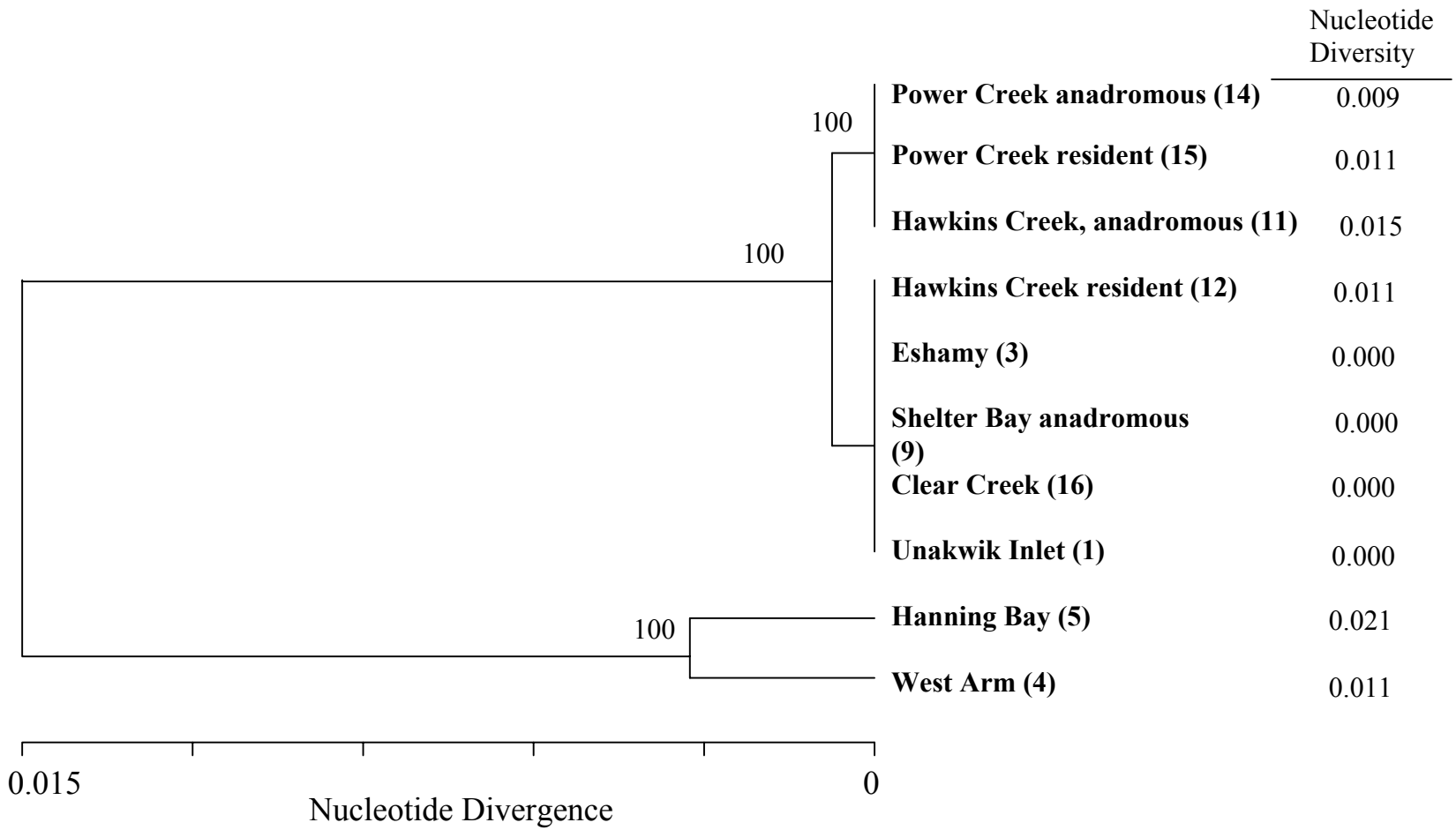


Figure 12. Genetic similarity of Dolly Varden from 10 locations in Prince William Sound based on mtDNA variation. Numbers at nodes are bootstrap (%) values based on 1000 simulations. Numbers in parentheses refer to locations in Figure 2.

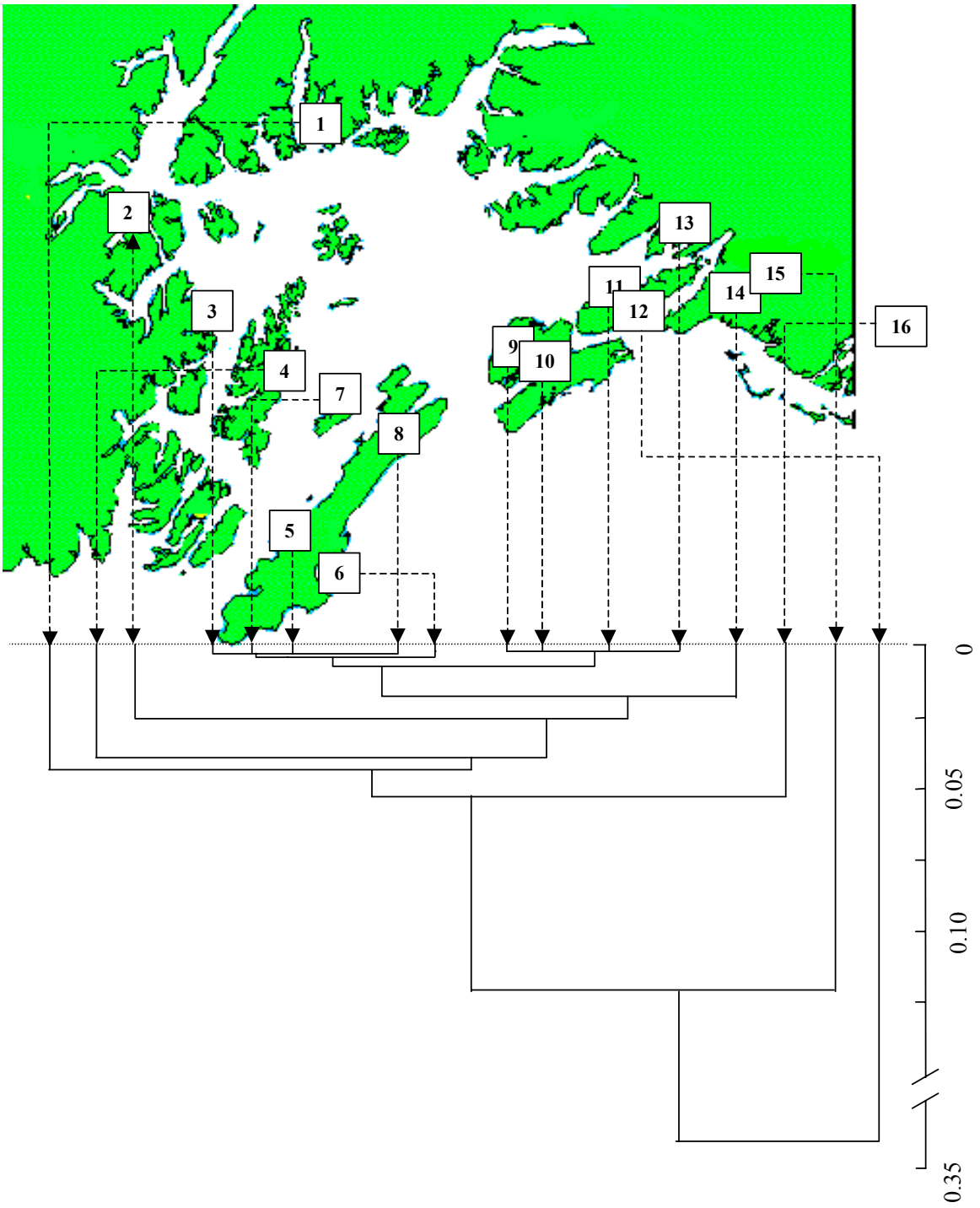
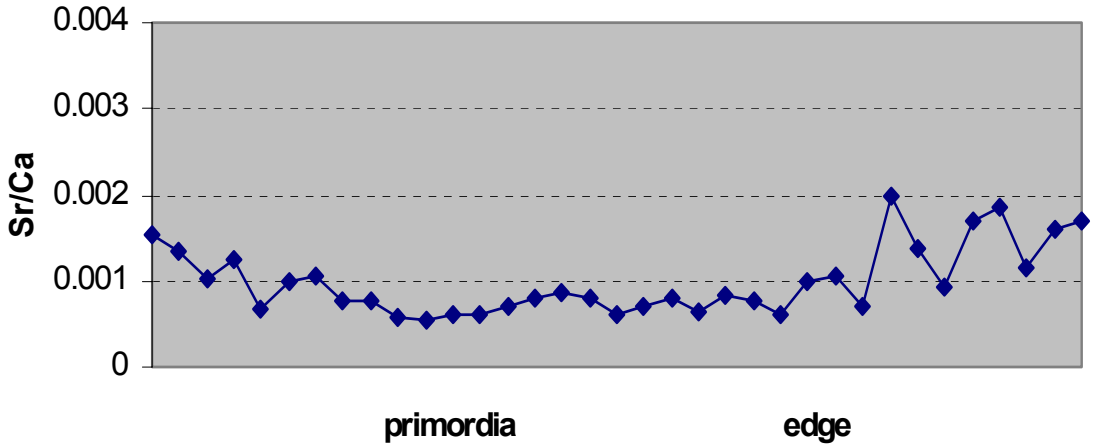
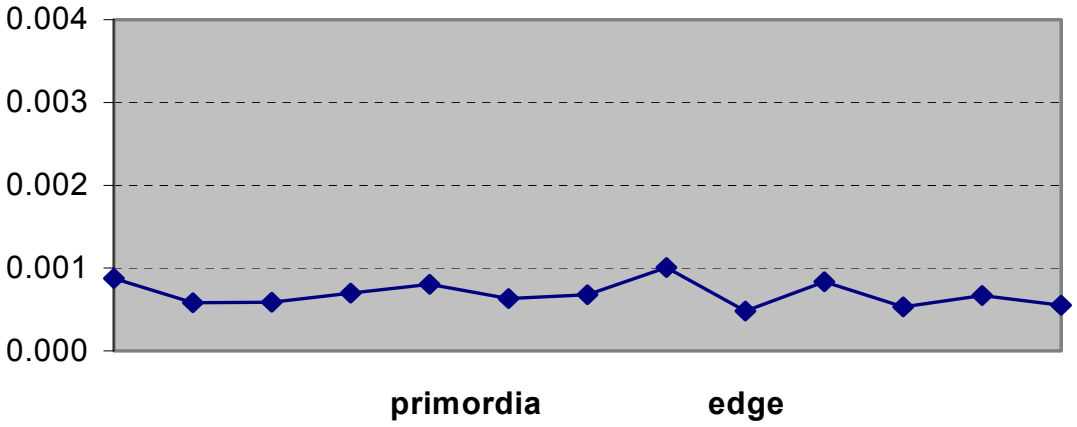


Figure 13. Genetic similarity of PWS Dolly Varden based allozyme and microsatellite DNA variation and coancestry distance.



(A)



(B)

Figure 14. Sr/Ca ratios in transects across the otoliths of Dolly Varden from (A) below barriers and (B) above barriers on Power Creek.

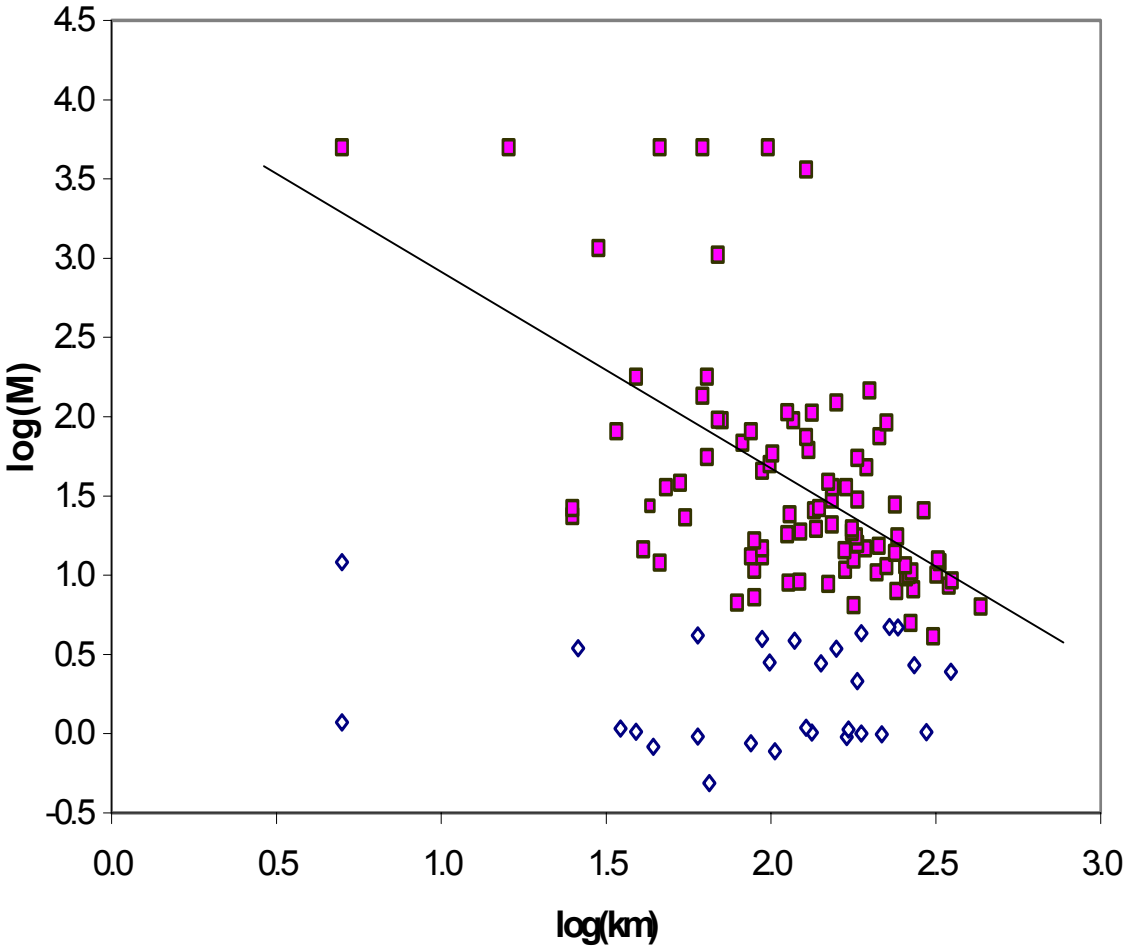


Figure 15. Relationship between gene flow ($\hat{M}$) and geographical distance for Prince William Sound Dolly Varden. Diamonds and squares are populations above and below putative barriers, respectively. Regression line: log ($\hat{M}$) = -1.24x + 4.14 with R^2 value of 0.33.

Pairwise estimates of migrants per generation, $\hat{M}$, for PWS Dolly Varden ranged from 0.49 to over 4000. $\hat{M}$ for above-barrier populations were almost always < 4 and showed no relationship to geographical distance, whereas all pairwise estimates of $\hat{M}$ for below-barrier aggregations were > 4 and decreased with increasing geographical distance (Figure 15). Largest values for $\hat{M}$, which strongly influenced the regression relationship between genetic exchange and distance, reflected nearly identical allele frequencies ($\theta \sim 0$) that occurred between above- and below-barrier populations in Shelter Creek and below-barrier aggregations in Hawkins Creek, Green Island, Port Chalmers, and Hanning Bay, however (Figure 15). Consequently, unlike cutthroat trout, isolation by distance relationships were not the same over all spatial scales (F = 125.72, p = 0.000). The decrease in $\hat{M}$ over distance was most pronounced at moderate distances (< 130 km) before reaching a constant of slope of –1.24 (Figure 8).

Discussion

Coastal Cutthroat Trout

All of our analyses pointed to the same conclusion about geographical genetic structure of coastal cutthroat trout in Prince William Sound. Geographically proximate aggregations around Orca Bay (Milton Lake and locations on Hawkins and Hinchinbrook islands) were genetically most similar and had high rates of genetic exchange, but genetic divergence increased in geographically more distant populations around Prince William Sound. The most divergent aggregations were those from northwestern PWS in Columbia Bay, Unakwik Inlet, Gunboat Lakes, and Green Island, which had the lowest levels of genetic exchange and genetic diversity (Figures 3-5, 7, 9).

We posed four *a priori* hypotheses for genetic differentiation of coastal cutthroat trout: (1) secondary contact after historical vicariance, (2) allopatric divergence of populations above and below barriers, (3) parapatric divergence associated with ecological differences natal freshwater habitats, and (4) isolation by distance. Our data do not support any of the first three hypotheses. Secondary contact after historical vicariance might have occurred if coastal cutthroat trout isolated in different glacial refugia had diverged allopatrically and then had recolonized PWS after the retreat of Pleistocene glaciers. Under this scenario, we might have expected to see a mosaic of populations from different genetic clades with possible zones of hybridization (Avise 1994). We found no evidence that genetic structure in coastal cutthroat trout in PWS was related to secondary contact after historical vicariance. In contrast, we concluded that coastal cutthroat trout most likely colonized Prince William Sound from a southern glacial refuge. All PWS populations belonged to a single mtDNA clade with nearly all individuals possessing the same haplotype. Estimated number of nucleotide substitutions between haplotypes in PWS was 0.0028, indicating relatively recent divergence of the two. The most common haplotype in PWS also occurred in coastal cutthroat trout from the Puget Sound, Washington, and the southern Oregon coast, although at much different frequencies and common alleles at allozyme loci in coastal cutthroat trout from PWS were the same as those in southern populations (Griswold 1996, Griswold 2002). Although extinctions and genetic drift could have erased any genetic signature of secondary contact, the most parsimonious explanation is that the cutthroat trout originated from a single ancestral source to the south. No cutthroat trout were known to have occurred north of PWS, although coastal cutthroat trout were one of many species that survived

glaciation in southern refuges and expanded into streams to the north (McPhail and Lindsey 1970, 1986).

Based on other studies (Currens et al. 1990, Griswold et al. 1997), we had hypothesized that allopatric differentiation of populations isolated above upstream barriers to migration could be an important component of genetic structure in coastal cutthroat trout. Above barrier populations may provide refuges for species and opportunities for evolution of novel phenotypic and genetic characteristics that buffer the survival of the species. We detected no significant differences between cutthroat trout above and below putative barriers in Shelter and Hawkins creeks, however. Likewise mean Sr/Ca ratios in the primordial region of otoliths from cutthroat below barriers in Shelter Creek were not significantly different. These results indicated that these putative barriers were either not barriers at all or they were only recently formed and may still have allowed downstream migration of coastal cutthroat trout. Genetically divergent populations of resident cutthroat trout may still exist above barriers in other parts of PWS, but we did not have the opportunity to study these.

We also found no evidence of parapatric differentiation associated with different freshwater natal environments (Table 8). We expected that under parapatric modes of differentiation, a continuously distributed population could develop geographical genetic differences through restricted gene flow (resulting from either philopatry or poor dispersal abilities) and natural selection for different environmental regimes (Endler 1977). It is possible the surrogates we used for environmental differences, such as stream substrate and ecoregional classifications, did not represent strong selection regimes that were acting on coastal cutthroat trout. Alternatively, selection regimes in PWS may be relatively homogeneous or recurrent extinctions and recolonizations of coastal cutthroat trout in response to a highly dynamic landscape may be overwhelming the opportunity for this kind of genetic structure.

The best explanation for the processes affecting genetic structure of coastal cutthroat trout that we studied in PWS is isolation by distance. We found a pattern of isolation by distance assuming that coastal cutthroat trout dispersed from east to west along the circumference of PWS, which approximates a one-dimensional stepping stone model for dispersal (Kimura and Weiss 1964), and assuming dispersal along the shortest possible shoreline distance, which allowed cutthroat trout to disperse along the northern, mainland shoreline. The results for the two were indistinguishable and suggest that dispersal of coastal cutthroat trout may follow a one-dimensional stepping stone model using PWS shorelines. Theory predicts that the slope of a one-dimensional stepping stone model is expected to be approximately –1.0; under a more complex two-dimensional stepping stone model, it will be approximately –0.5 (Slatkin and Maddison 1990, Slatkin 1991). In our data, the correlation between migrants and distance for the former was significant at $p = 0.009$ with a slope of –0.84, where as the latter was significant at $p = 0.015$ with a slope of –0.88. The 95% confidence intervals for the slopes overlapped and both included –1.0 but not –0.5. In contrast, our results did not support dispersal of coastal cutthroat across large, open water of PWS, which is consistent with other studies. Jones and Seifert (1997) noted that coastal cutthroat trout tended to move along shorelines and estuaries. In PWS, recaptured coastal cutthroat migrated an average of 2 km from the original tagging location (Bernard et al. 1995). In Hood Canal, Washington, coastal cutthroat trout crossed open waters, but these distances were generally less than 5 km (Wenburg and Bentzen 2001).

Overall, our results suggested that gene flow and genetic drift were approximately at equilibrium in PWS coastal cutthroat trout, but that recent colonizations may be resetting long-term patterns of isolation by distance. In non-equilibrium populations, such as those in recently colonized habitats, the inverse relationship between $\hat{M}$ and geographical distance is first detectable at smaller spatial scales and depends on the parameter $\sqrt{2Nm\tau}$, where N is the population size, m is the proportion of migrants each generation (pairwise $Nm = \hat{M}$), and τ is the time since the population was founded (Slatkin 1993). Consequently, for recently founded populations (small τ), isolation by distance is first detected at short distances before it spreads over large spatial areas. If, however, isolation by distance is detected in recently colonized systems (small τ) with low migrants per generation (Nm), then the pattern more likely reflects founding events rather than contemporary demographic processes. Our data showed a constant negative slope of the regression of $\hat{M}$ on geographical distance over the entire spatial scale of PWS (Figure 8), which indicated long-term equilibrium of gene flow and genetic drift. Because isolation by distance over this spatial scale takes time, we concluded that coastal cutthroat trout are not recent colonizers of Prince William Sound.

A revealing anomaly occurred at distances between 90-150 km, however. This spatial scale includes aggregations of coastal cutthroat trout at the most northern edge of the species range in northwestern PWS where genetic exchange (Nm or $\hat{M}$) is lowest (Figure 7). It also includes Columbia Bay, Unakwik Inlet, and perhaps other aggregations that were only recently colonized by coastal cutthroat trout (small τ) as tidewater glaciers receded during the last 350-150 yrs (Post 1975). Theory predicts that with low numbers of potential colonists and stepwise dispersal, founder events would occur with each colonization leading to decreased allelic diversity and increased divergence in recently founded populations (Le Corre and Kremer 1998). This is indicated in our data, which showed that cutthroat trout aggregations in this region had lower levels of allelic diversity and heterozygosity (Figures 3, 4) and greater divergence (Figure 5). In addition, at this spatial scale, slopes of the isolation by distance regressions peaked at –1.02 and then decreased with increasing distance to a slope that remained constant over the whole PWS (Figure 8), which is a pattern is similar to that of non-equilibrium populations approaching isolation by distance. Consequently, the pattern of isolation by distance at this spatial scale in PWS most likely reflected both founding events based on source populations already differentiated by isolation by distance and current demographic processes as populations reset to a new isolation by distance equilibrium.

Our conclusions about the population differentiation in PWS coastal cutthroat trout were strengthened by Bayesian cluster analysis (STRUCTURE; Pritchard et al. 2000), which identified population structure that minimizes heterozygote deficiency and linkage disequilibrium in the data. Heterozygote deficiencies are relatively common in fishes (reviewed in Waldman and McKinnon 1993; see also Castric et al. 2001) and deviations similar to ours have been documented in microsatellite variation in coastal cutthroat trout (Wenburg and Bentzen 2001). Although we cannot completely rule out analytical artifacts, such as "null" alleles (Callen et al. 1993) or small allele dominance (Wattier et al. 1998) in our data, we hypothesized that Walhund effects, or the mixing of differentiated subpopulations (Wahlund 1928), better explain the deficiencies we observed. Other biological explanations, such as matings of close relatives (Wright 1921) or non-random sampling of fish from limited families

(Pudovkin et al. 1996), seem unlikely because we attempted to sample larger populations at life history stages where family members should be well dispersed. In our case, the life history of coastal cutthroat trout (foraging migrations to non-natal streams), the locations where samples had to be collected (near tidewater), and the consistent lack of heterozygotes in both allozyme and microsatellite DNA data all strongly suggested mixed aggregations. In addition, no set of loci was especially prone to Hardy-Weinberg deficiencies and there was no geographical pattern to the deficits. Consequently, if Wahlund effects existed in our data, we expected that population structure identified by STRUCTURE analyses should be similar to that from other analyses, whereas if the deficiencies were because of technical artifacts they would confound geographic patterns. We found the same population structure using STRUCTURE as other analyses: geographically proximate aggregations around Orca Bay had the greatest amount of mixing, but isolation and genetic divergence increased in geographically more distant populations around Prince William Sound. The most divergent aggregations were those from northwestern PWS in Columbia Bay, Unakwik Inlet, Gunboat Lakes, and Green Island (Figure 9). The contributions of Columbia Bay and West Arm cutthroat trout to Orca Bay aggregations in this analysis, however, were not reflected in other analyses and may have reflected STRUCTURE's assumption of an equal probability of migration from every aggregation. This would not account for the biologically more reasonable assumption that large geographical distances lessen the probability of contributions.

Dolly Varden

Despite similarities in their life history, Dolly Varden in PWS had different patterns of genetic variation than coastal cutthroat trout. Aggregations below barriers were characterized by large amounts of genetic exchange and minor genetic differentiation. Median genetic exchange among below-barrier aggregations was 24 migrants per generation (Figure 15), for example, and values for θ_S based on microsatellite and allozyme variation were 0.034 and 0.044, respectively (Table 6). We saw no strong geographical patterns of differentiation. Mitochondrial DNA variation ($\theta_S = 0.35$), however, suggested much more limited exchange among some below-barrrier populations (Table 6) and differences between above- and below-barrier populations were pronounced in Dolly Varden.

We posed four non-exclusive *a priori* hypotheses for genetic differentiation of coastal cutthroat trout: (1) secondary contact after historical vicariance, (2) allopatric divergence of populations above and below barriers, (3) parapatric divergence associated with ecological differences in natal freshwater habitats, and (4) isolation by distance. Our results indicated that allopatric divergence of isolated Dolly Varden above barriers was a major component of the genetic diversity in PWS Dolly Varden. The other three modes of differentiation had less support.

Mitochondrial DNA variation did not provide convincing evidence of secondary contact after vicariance. We concluded that Dolly Varden most likely colonized PWS from a northern glacial refuge, although we hope future work will reexamine this conclusion. Dolly Varden most likely colonized deglaciated streams of the northwestern North American after surviving Pleistocene glaciation in Beringia (McPhail and Lindsey 1970), a glacial refuge north of PWS, and in a southern glacial refuge (Redenbach and Taylor 2002). Redenbach and Taylor (2002) identified areas of secondary contact between the northern and southern forms in deglaciated areas of British Columbia using a diagnostic fragment length-polymorphisms in ND-1 restricted with

Hae III. They suggested that areas to the north, including PWS, would have been colonized from the northern refuge. The three fragment length-polymorphisms at ND-1 digested with *Hae III* that we identified in PWS that were most common pattern in our data was typical of the northern clade (550-, 380-, 240-, 200-, 190-, and 100-bp) of Redenbach and Taylor (2002; Eric Taylor, University of British Columbia, Vancouver, B.C., personal communication). Haplotype ACA, which was much less common (Table 9), was similar to the diagnostic pattern for the southern clade (550-, 490-, 300-, 200-, 190-, and 100-bp). Evolutionary distance between haplotypes in PWS, $\hat{d}$, ranged from 0.015 to 0.037, indicating a relatively long history of divergence. We suspected that haplotypes found in PWS Dolly Varden and perhaps those populations studied by Redenbach and Taylor (2002) most likely existed in refugial populations of Beringia and that frequencies in recolonized areas or other refugia may have been profoundly affect by genetic drift. Had Dolly Varden recolonized PWS from southern refugia, we would have expected populations south of PWS to have similar frequencies of the southern clade haplotype. In contrast, Redenbach and Taylor (2002) found no populations of Dolly Varden between zones of secondary contact in British Columbia and PWS with such frequencies. They did note, however, that persistence of southern clade haplotypes in low frequencies in a few more northernly streams suggested that this form may have persisted in Beringia.

We found no evidence of parapatric divergence associated with ecological differences in natal freshwater habitats based on allozyme and microsatellite DNA variation. Significant mtDNA differences existed between Dolly Varden from eastern and western streams and between aggregations from island and mainland ecosections, however. Both these differences were due to the high frequencies of alternate haplotypes in Dolly Varden from West Arm and Hanning Bay, which were both western and island habitats. These differences might simply reflect sampling error because of low sample sizes (Table 9). If, however, they represented genetic differences among aggregations, it raises the possibility of sexual dimorphism in migratory behaviors of Dolly Varden. Because mtDNA variation is maternally inherited (Gyllensten et al. 1991), it reflects migration and genetic drift among female lineages, whereas allozyme and microsatellite DNA variation reflect patterns for both sexes. Values for θ_S for mtDNA variation in PWS Dolly Varden were nearly an order of magnitude larger than values for allozyme and microsatellite DNA variation, indicating potentially much greater philopatry for females than males. Sex-based dispersal may occur where there is strong competition for mates or other resources (Greenwood 1980, Perrin and Mazalov 2000). Theoretically, sexual dimorphism in migration could lead to differences in allele frequencies among sexes and subsequent heterozygote excesses among randomly mating aggregations (Prout 1981), which we did not see, but low genetic variance among sexes or nonrandom mating could have prevented such excesses. Interestingly, male rivers otters (*Lontra canadensis*) from this same area of PWS had much higher rates of dispersal and gene flow among geographically close populations than females (Blundell et al. 2002).

Unlike coastal cutthroat trout, Dolly Varden did not show strong isolation by distance. Patterns of isolation by distance among below-barrier aggregations occurred only at shorter distances (< 100 km) with large levels of genetic exchange (Figures 8, 15). This indicated that high rates of gene flow were preventing equilibrium with genetic drift and isolation by distance was not leading to strong population differentiation. In contrast, low levels of genetic exchange among populations above barriers (Figure 15) and lack of isolation by distance indicated that these

barriers were largely preventing migration and the populations were diverging without respect to geographical distance.

Isolation and divergence of above-barrier populations may be important sources of new phenotypes and adaptation for species over long time periods if these populations can persist in the face of environmental disturbances and contribute to colonization. Overall, differentiation was nearly twice as large for above- and below-barrier populations ($\theta_S = 0.101$) than for only below-barrier aggregations in PWS ($\theta_S = 0.040$, Table 8), indicating that this was an important source of genetic diversity in Dolly Varden. As our results show, the pattern of differentiation in above- and below-barrier aggregation would not be predictable without knowing the degree of isolation and population history. In two of the three comparisons, we identified significant differences and reduced genetic diversity in above-barrier populations (Figures 10, 11) but not in a third. This may be because the barrier was recent and effective population sizes were large or because the barrier was more permeable than expected.

Differences Between Coastal Cutthroat Trout and Dolly Varden

Although coastal cutthroat trout and Dolly Varden have similar life histories, we expected different genetic structures in the two species because of their different biogeographical histories. Compared to coastal cutthroat trout, Dolly Varden might have had longer to evolve differences among populations because they were probably among the first colonizers of recently deglaciated streams (Milner et al. 2000, Milner and York 2001) in PWS and were near the center of the species range as it radiated out from Beringia. In contrast, coalescence times for coastal cutthroat trout following colonization of PWS would presumably have been much less than for Dolly Varden because they may have arrived later, although geographical isolation and presumably suboptimal environments affecting small population sizes could still lead to genetic divergence (Carson and Templeton 1984). To reach the periphery of the species range in PWS, coastal cutthroat trout had to migrate northward as glaciers receded along the Pacific coast from refugia in southern British Columbia or Washington (McPhail and Lindsey 1986), despite their presumably limited dispersal ability. Lack of isolation by distance across much of their range (Wenburg et al. 1998; Thomas Williams, NOAA Fisheries, Santa Cruz, CA, unpublished data), except at small spatial scales (e.g., Wenburg and Bentzen 2001) may be evidence of such limited dispersal abilities. Because the spatial scale for divergence by isolation by distance depends on $\sqrt{2Nm\tau}$, we might expect Dolly Varden to show isolation by distance but not coastal cutthroat trout.

Given this history, our findings that coastal cutthroat trout diverged over a large geographical area through isolation by distance—whereas Dolly Varden did not—were surprising. The best explanation may be that coastal cutthroat trout showed isolation by distance over this spatial scale in PWS but not elsewhere because they maintained strong migratory capabilities that allowed genetic exchange and because peripheral environments were instable but not necessarily suboptimal. Although coastal cutthroat trout showed limited dispersal in other areas, in PWS genetic exchange ranged from 0.59 among newly colonized habitats to 208 migrants per generation. This ability to disperse may reflect the predominance of amphidromous behavior of these fish compared to more southerly populations where loss of amiphidromous life history forms has led to efforts to protect coastal cutthroat trout (Johnson et al. 1999). In regions such as PWS where cold, nutrient-poor glacial streams drain into productive marine environments, the

increase in individual growth, fecundity, and reproductive success may outweigh physiological costs of switching between hypo- and hyperosmotic environments (Thorpe 1987, Maekawa and Nakano 2002). This may partially explain why the only two places where coastal cutthroat trout are known to have diverged through isolation by distance were in PWS and Hood Canal (Wenburg and Bentzen 2001), a highly productive fjord of Puget Sound, Washington, that is fed by glacial streams of the Olympic Mountains. Additionally, freshwater habitat instability, which is part of the dynamic landscape of PWS, strongly selects for dispersal over residency (Clobert et al. 2001). Suboptimal environments, however, could increase extinction rates or limit population sizes to levels that prevent isolation by distance. Consequently, although coastal cutthroat trout in PWS were at the periphery of the range, the habitat may have been optimal for life history strategies that allowed the species to reach equilibria between gene flow and genetic drift.

Dolly Varden, despite potentially long coalescence times, did not diverge through isolation by distance because gene flow overwhelmed genetic drift. This may simply indicate that Dolly Varden had high dispersal abilities and were not as philopatric as other salmonids. Although Dolly Varden are generally thought to return to natal stream to reproduce (Bernard et al. 1995), they will cross deep, open waters and migrate large distances (McCarron and Hoffman 1993), which provides opportunity for colonization and gene flow. We detected significant correlations between genetic divergence and geographical distance regardless of whether we assumed geographical distances followed shorelines or went straight across PWS. This suggested that open water migration was not a barrier for Dolly Varden. Dolly Varden captured in the Beaufort Sea, Alaska, for example, included significant proportions from geographically distant streams (Krueger et al. 1999).

Lack of differentiation may also indicate that Dolly Varden were more affected by local extinctions in the highly dynamic landscape of PWS than coastal cutthroat trout. Dolly Varden were often the first fish to colonize deglaciated streams (Milner et al. 2000), before streams became geomorphically stabilized by forested riparian areas, large wood, and deep pools. Although this life history strategy allows them to find unoccupied habitat, it may also make them more vulnerable to local extinctions. Frequent local extinctions and recolonizations are commonly forgotten as a source of gene flow (Slatkin 1985). Where probability of local extinction, e_o, is small and $m \leq e_o$, m can be replaced by $m + (e_o/2)$ in models of population differentiation (Slatkin 1977). Consequently, even if m is very low, if e_o is high enough, little differentiation will occur among local aggregations. If both occur, gene flow may continually overwhelm genetic drift, preventing isolation by distance over large areas even if many generations have passed since a species radiated into an area.

Recovery Programs

The similar life histories of Dolly Varden and coastal cutthroat trout in PWS indicate that recovery programs for populations that were injured in the spill should have similar components. The emphasis on the components for each of the species should vary, however, to reflect the differences in distribution, migration, genetic structure, and population sizes between the two species. Here, we focus on five aspects of recovery programs: (1) status assessments, (2) habitat protection, (3) habitat restoration, (4) harvest regulations, and (5) population reintroductions or artificial propagation.

The status of each species should be determined before restoration programs are undertaken and at regular periods during the recovery. A key element is compiling a thorough assessment of the current distribution of each species and the available habitat, including populations above barriers. In the beginning of our study, we found few systematically collected data were available on the distribution of coastal cutthroat trout, for example. An efficient recovery plan depends on these data. Our data on the relationship between independent populations (those with $\hat{M} < 1$, for example), migration, and geographical distance could be used to quantitatively assess potential for natural recovery, colonization, or extinction of injured populations, if distributions of healthy local populations and proportions of occupied and occupied suitable habitat were known (Slatkin 1985). This information would also allow comparisons of injured populations with non-injured populations that are in the same general area, as recommended by the EVOS Trustee Council. This would refine the initial status assessments following the oil spill, which concluded that populations of each species were injured by the spill based on comparisons with populations in eastern PWS (Hepler et al. 1993). These areas, which may have different climates and nearshore conditions, also had different population growth rates (G.H. Reeves, unpublished data). Variation in growth rates among populations in each area were relatively small, which suggests that it would be possible to use non-oiled sites in the same area as controls. The small variability in growth rates also eliminates the need to have pre-oil spill growth rates to determine if injured populations have recovered.

Recovery of injured populations depends on protection and restoration of both freshwater and nearshore environments. Freshwater environments are especially important for two reasons. First it is obligatory habitat for both species, whereas the marine environment is not. Adults of both species spawn and over-winter in freshwater. Juveniles rear there for up to four years before moving to the marine environment. Second, Protection and recovery of freshwater habitats may increase growth rates and survival in freshwater that at least partially counteract impacts of the oil spill and that offer the most immediate and cost effective means of assisting the recovery of injured populations. Freshwater environments of injured populations were not directly impacted by the spill, whereas near-shore marine and estuarine habitats used by these fish were damaged and may take longer to recover

Protection of freshwater habitat is more efficient than recovery of damaged habitat. Given the uncertainty of success of restoring damaged habitat (Reeves et al. 1991), protection should be the primary focus of this component of a recovery program. Cutthroat trout and Dolly Varden are vulnerable to the degradation and alteration of freshwater habitats, particularly loss of pools and decreases in habitat complexity, that can result for land management activities such as timber harvest and associated road building (Reeves et. al 1993, 1995). They also may spawn in small headwater streams that are given little or no protection under forest practices rules. Based on our results, priorities for habitat protection for coastal cutthroat trout should focus (1) on watersheds with well-developed cutthroat trout habitat and healthy populations adjacent (within 30 km shoreline distance) to injured populations, (2) well-developed cutthroat habitat with injured populations or unoccupied habitat adjacent to healthy populations, and (3) other healthy populations. Prioritizing good habitat adjacent to healthy or injured populations may allow genetic exchange or infusion of 15 migrants per generation (actual census size will probably be 2-5 times greater) for colonization and population rebuilding. For Dolly Varden, watersheds with injured populations should be the priorities for protection, as long as healthy populations are

not simultaneously reduced. Populations above barriers are a second priority. Based on our data, dispersal rates of Dolly Varden are high and not necessarily limited to shorelines, which should allow for adequate colonization and genetic exchange from a variety of sources. Above barrier habitats are important because populations above barriers are the major source of the evolutionary diversity that allows the species to adapt to new challenges. Habitat protection should focus either on outright purchase of watersheds and limiting or eliminating activities in the watershed; or, protection could be in the form of the purchase of conservation easements in riparian areas in watersheds on non-public lands that currently have good habitat conditions.

Restoration activities should be directed towards increasing habitat complexity. Habitat complexity should help maintain the equilibrium between gene flow and genetic drift that exists in PWS coastal cutthroat trout. It may also encourage subpopulation differentiation in Dolly Varden, if the high rates of gene flow in Dolly Varden reflect frequent local extinctions. For coastal cutthroat trout, restoration efforts should be directed at creating deep pools (i.e., >1 m) with complexity formed by pieces of large wood (Reeves et al. 1995). Likewise, density of juvenile Dolly Varden is related to habitat complexity; density decreases as amount of woody debris decreases (Dolloff 1986, Elliott 1986). Complexity can be increased immediately by the addition of large wood. At the same time, riparian zones should be restored to provide future sources of wood. Removing roads and culverts should also be part of the any restoration program.

Harvest has a direct impact on population abundance, population growth, and dispersal. We recommend that harvest of coastal cutthroat trout in PWS should be restricted in all areas and possibly eliminated in areas with injured populations. Cutthroat trout populations, especially in western PWS, were relatively small (McCarron and Hoffmann, 1993). Coastal cutthroat trout can also be relatively easy to catch with sport fishing gear and fishing pressure may increase as access to some of populations becomes easier with road development, such as between Anchorage and Whittier These factors, combined with our findings that population exchange were lowest in western PWS, indicate that these coastal cutthroat trout populations may be very vulnerable to overharvest, which could impede recovery of populations damaged by the oil spill. In contrast, regulation of harvest for Dolly Varden does not need to be as widespread or extensive as it does for coastal cutthroat trout. Dolly Varden were abundant throughout PWS (McCarron and Hoffmann 1993) and large populations may better accommodate harvest. Also, less fishing pressure may be directed at Dolly Varden compared to coastal cutthroat trout. Regulations could include bag and seasonal possession limits and gear restrictions, with more severe restrictions on injured populations.

Rebuilding injured populations through transfers from other streams or artificial propagation is not necessary to recover injured populations of either species. Based on our data, significant genetic differences existed among coastal cutthroat trout populations in PWS, but migration rates were high enough between adjacent populations to allow for recolonization. If transfers are needed, they should occur between nearby aggregationsl. Because abundance of coastal cutthroat trout tended to be low, however, removing large numbers from a healthy population for transfers could have negative impacts on the donor population. Likewise, our data indicated that Dolly Varden aggregations were genetic similar and exchanged large numbers of migrants. Abundances of Dolly Varden populations were also larger than cutthroat trout. Given these

factors, Dolly Varden appear to have a better inherent potential to recover when numbers decline than do cutthroat trout.

Opportunities for and the potential of resident populations to contribute to the recovery of injured anadromous populations appear to be limited in PWS. In coastal cutthroat trout, aggregations above barriers were indistinguishable from those below barriers. Griswold (1996) examined the genetic relationship between resident and anadromous coastal cutthroat trout in a watershed in southern Oregon and in southeast Alaska and found that resident and anadromous forms in southeast Alaska were similar to each other, whereas in Oregon, some resident populations were similar to anadromous populations and others were very different. In PWS, many barriers that separated anadromous and resident populations of each species have been modified. The consequences of eliminating the isolation of the two forms are unknown, but it is most likely that genetic differences have been reduced.

We expect that recovery of injured coastal cutthroat trout populations will be slow relative to Dolly Varden. Cutthroat trout populations were characterized by greater geographical isolation, genetic divergence, and small population sizes. These factors suggest that recovery of injured populations depends less on nearby populations than on the productivity of the population and the extent that it was injured by the oil spill. In contrast, injured Dolly Varden population should recover more quickly than injured coastal cutthroat trout populations, because higher rates of immigration should contribute to the recovery process. The primary factor determining the rate of recovery of damaged populations of both species will likely be the recovery of the environment. Once environmental conditions improve, injured populations should be expected to recover relatively quickly.

Conclusions

This study provided a unique opportunity to examine and compare the life history and genetics of two salmonid species that co-occur in the wild. Our results suggested that coastal cutthroat trout populations were small and isolated but genetically stable, except in the north and western parts of PWS. There we found evidence of greater isolation and reduced genetic diversity indicating that they had recently colonized parts of that area. In contrast, we found that the biggest genetic differences in Dolly Varden occurred between above- and below-barrier populations. Below-barrier populations, however, showed high rates of genetic exchange and strong genetic similarity. These findings reflected the different behavioral and demographic strategies that the two species have for surviving in a highly dynamic landscape. Recovery programs need to consider these strategies to be most effective.

Acknowledgments

We would like to acknowledge the assistance of Dave Schmid, Ken Hodges, Merlyn Schelske, Dave Saiget, and Sam Greenwood and numerous volunteers of the USFS Cordova Ranger Station for assistance in selecting study sites and collecting samples. We would also like to acknowledge George Covell for his insight into coastal cutthroat distributions in PWS and his helpful advice in selecting study sites. Andy Hoffman, Alaska Department of Fish and Game, Anchorage, AK, provided assistance in obtaining collection permits and selecting study sites. Ken Holbrook offered help and advice with the preparation of proposals and annual reports. Dave Gibbons initially encouraged us to undertake this work. We would like to thank Brenda Wright for collecting Dolly Varden samples from Prince of Wales Island. We would also like to acknowledge the expertise of Janet Hanus of the Oregon Cooperative Fishery Unit, Corvallis Oregon in developing the protocol for microsatellites. We thank Marcos Tovar for his help in the laboratory. We would also like to acknowledge our cooperators at the Washington Department of Fish and Wildlife, and Anne Marshall in particular for analyzing Dolly Varden allozyme variation.

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