POPULATION GENETIC STRUCTURE OF

ALASKAN PACIFIC OCEAN PERCH (*Sebastes alutus*)

By

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A

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Abstract

Knowledge of the population structure of a species is essential for its effective management and sustained production. Although Pacific ocean perch (*Sebastes alutus*, POP) is an important species both economically and ecologically, little is known about its population structure and life history in Alaskan waters. The objectives of this study were to describe the population structure of POP in terms of the numbers and geographic scale of local populations, their connectivity, and the compatibility of that structure with current management. Fourteen microsatellite loci were used to characterize the population structure genetically in eleven geographically distinct collections from sites along the continental shelf from the Queen Charlotte Islands to the Bering Sea. In spite of the many opportunities for most life stages to disperse, there was strong geographically related genetic structure (F_{ST} =0.0123, $p < 10^{-5}$). Adults appear to belong to neighborhoods that exchange genetic information at relatively small spatial scales (14 to 90 km). Although this suggests limited movement, connectivity is evidenced by the isolation-by-distance relationship, the apparent northwestward movement of gene flow in the Gulf of Alaska (GOA), and the break in geneflow in the central GOA. The observed population structure has a finer geographic scale than management areas, which suggests that current fisheries management should be revisited.

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Introduction

The Pacific ocean perch (*Sebastes alutus*, POP) is one of more than 100 species of rockfishes worldwide, most of which are distributed in the northern Pacific Ocean. Rockfishes are an ecologically diverse and economically important group of marine fishes; consequently, their conservation is of interest to fisheries managers (Lyubimova 1963, Westrheim 1975, Love et al. 2002). Pacific ocean perch, the most abundant rockfish in Alaskan waters, occurs along the Pacific Rim, ranging south to California and west to Japan. They have been fished extensively throughout their North American range since the 1940's. Like many rockfish species, POP are long-lived and late-maturing (Paraketsov 1963, Gunderson 1977, Lunsford 1999, Hanselman et al. 2003, Spencer and Ianelli 2005). These life history characteristics and the general lack of knowledge regarding their population structure make POP, and most rockfishes, difficult to manage for commercial harvest.

Although the biology of some species of rockfish have been well studied, relatively little is known about the life history characteristics or population structure of many species distributed in Alaskan waters, including POP. Much of the life history information for POP was obtained from studies of British Columbia stocks (Gunderson 1971, 1972, 1974, Leaman and Kabata 1987, Leaman 1991). Pacific ocean perch are viviparous; consequently, knowledge of the timing and locale of larval release and the distances larvae and juveniles disperse is essential in understanding their distribution, life history, and critical habitats (Moser and Boehlert 1991). After insemination females may migrate into deep water (500-700m) where they stay for the winter months until larvae

are released in the spring; however, the precise locations and depths are not known (Gunderson 1972, Love et al. 2002). Because their larvae are planktonic for several weeks to a few months before they settle as juveniles, oceanic currents could play a role in their dispersal and survival (Carlson and Haight 1976, Ainley et al. 1993). Adult aggregations, composed of fishes age 6 and higher, are commonly found on shelf/slope or shelf/gully breaks along the continental shelf, are planktivorous, and are generally semi-demersal (Carlson and Haight 1976, Gunderson 1977, 1997, Scott 1995). More studies are needed to understand population distribution, location of critical habitats throughout the POP life span, and the times of year when these habitats are necessary for survival.

The life history of POP and past fishing records indicate that they, like many rockfishes, are vulnerable to over harvesting. During the 1960's and early 1970's, POP were intensely targeted by a foreign trawl fishery, which was composed mostly of Japanese and Soviet vessels (Ito 1986). Between 1967 and 1984, Alaskan POP stocks were reduced by 80% from virgin biomass estimates throughout their range (Gunderson 1977, Ito 1986, Hanselman et al. 2005, Spencer and Ianelli 2005). The domestic fishery took over in 1985 and continued to grow until 1991. From 1991 to 1996, the fishery was restructured and management practices were changed to encourage the rebuilding of POP stocks (Ianelli and Heifetz 1995, Hanselman et al. 2005). Since 1996, catches of POP have increased and there is evidence for increased recruitment and biomass. However, characterization of their population structure and life history is still necessary to understand their demography, which is the basis of any meaningful management design (Hanselman et al. 2005)

Detecting population structure in marine species can be challenging. Many populations do not have obvious physical boundaries and the limiting biological or ecological factors may be difficult to observe. In addition, many species have the opportunity and ability to disperse long distances as larvae, juveniles, or adults. Many previous studies in the marine realm have been unable to detect population structure, and other studies detected only weak structure (Gold et al. 1994, Palumbi 1994, Garoia et al. 2004). Demographic attributes, such as large population sizes and complicated age structures, and life history characteristics, such as planktonic larvae and mobile adults, may explain the weak population structures observed in some species (Waples 1987, Gold et al. 1994, Palumbi 1994). In addition, the genetic tools that have been used in many previous studies have had low resolution. One of the consequences of the inability to detect population structure is the possible erroneous inference by many fisheries scientists that most marine organisms disperse widely and are genetically panmictic.

Even in a continuously distributed species, population sub-structure can occur if the average movement of an individual between birth and reproduction is much less than the species range. When movement is limited, genetic exchange will only take place among individuals in close geographic proximity to each other, which creates genetic structure within the species range. Consequently, genetic divergence over geographic distance may reflect the geographic scale of the population structure (Wright 1943, Matala et al. 2004a, 2004b). The existence of structure in commercially harvested marine fishes that have been presumed to be panmictic populations may expose them to overfishing and reduced productivity.

In large marine populations, limited dispersal may not result in substantial population divergence, as measured by parameters such as F_{ST}, which is a standardized measure of genetic divergence among populations (Wright 1956, Weir and Cockham 1984). Consequently, low levels of divergence (small F_{ST}s) do not necessarily signal lack of genetic isolation or structure, and may be consistent with restricted demographic exchange (Stepien 1999, Palumbi 2003). Currently used genetic markers can reveal small departures from panmixia, even in large marine populations. Recently developed biochemical genetic markers, such as microsatellite analysis, provide tools that can increase the resolution of population structure (e.g., Ruzzante et al. 1998, Roques et al. 1999, 2002, Withler et al. 2001, Buonaccorsi et al. 2002, Olsen et al. 2002, Cope 2004, Matala et al. 2004a, 2004b, Saillant and Gold 2006). Research conducted with microsatellites has uncovered evidence for finer-scale structure than was previously recognized in a number of species, including rockfishes. However, many of the analytical methods that are used to estimate characteristics of population demographics from genetic data yield undependable results when applied to species that have low levels of divergence (Waples 1998, Latch et al. 2006, Waples and Gaggiotti 2006). Even so, these results can provide some useful insights if the assumptions underlying the statistical tests are considered and an array of parallel analyses are used to evaluate the genetic data. For many marine populations, genetic analysis is the only practical method to investigate population structure and its underlying demographics for use in effective fisheries management.

An allozyme study in the mid-1980s proposed that Alaskan POP were genetically similar throughout their range, but a weak geographic cline observed at a single locus suggested that population structure might be present but that better tools would be required to resolve it (Seeb and Gunderson 1988). This structure could be explained if adults remain in a limited area and larvae that successfully recruit do not move far from their natal region even when there are opportunities to disperse (Stepien 1999, Buonaccorsi et al. 2002, 2004, Taylor and Hellberg 2003). Microsatellite studies have also been used to characterize rockfish life history, determine effective management scales, estimate gene flow, and hypothesize patterns of dispersal in larvae (e.g., Roques et al. 1999, 2002, Rocha-Olivares and Vetter 1999). A recent study of POP that was based on microsatellite variation reported genetically distinct populations in British Columbia within a small geographic area (Withler et al. 2001). However, the results of this study cannot be extrapolated to stocks of Alaskan POP, because the structure of the British Columbia populations occurred in a restricted area and appeared to be associated with the prevailing currents within this area.

In this study I used microsatellites to explore the population structure and resultant demographic implications for management of harvest of POP in Alaskan waters. The specific questions I asked were: (1) Is geographic population structure detectable in Alaskan waters? (2) If divergence or structure exists, what is the nature of this structure and its geographic scale and how are the populations connected? (3) Did historic influences, such as the large decline in POP abundance, leave a genetic signature that can be detected? and (4) Is the population structure observed compatible with current

management? Previous studies of POP and other Alaskan rockfishes reported low F_{ST}s, consequently, I used multiple parallel statistical procedures, when available, to address these questions.

Materials and Methods

Sample Collection and DNA Isolation

Alaskan POP samples were obtained opportunistically by National Marine Fisheries Service (NMFS) Auke Bay Laboratory personnel on trawl surveys in 1999, 2000, 2002, 2003, and 2005. Tissue samples were taken from fish caught in the Bering Sea and the Gulf of Alaska (GOA). The numbers of fish sampled at any one site ranged from 15 to 100 (Table 1, Figure 1). Collections were pooled geographically for analysis into eleven "populations": Queen Charlotte Island (QCI), Cross Sound (CSS), Yakutat (YAK), Cordova (CORD), Kodiak (KOD), Shumagins (SHU), Akutan (AKU), Central Aleutians (ALE), Western Aleutians (WAL), Southern Bering Sea (SBS), and Central Bering Sea (CBS). The geographic areas represented by some of the pools included multiple trawls, but all collections pooled into a "population" were caught within a radius of 100 km and all, except SBS, were caught in the same year (Table 1).

Tissue was dissected from specimens, placed in DNA preservative (Seutin et al. 1991), and stored at -20°C. Total genomic DNA was isolated from the tissue samples using the Puregene DNA™ (Gentra Systems, Minneapolis, MN) isolation protocol. DNA was rehydrated with 1X TE (0.01M Tris-HCl, 0.001M EDTA, pH 8.0) and stored at -20°C until it was analyzed.

Table 1. Location and dates of Pacific ocean perch (*S. alutus*) sample collections. Collections were grouped geographically into "populations" based on geographic proximity (*n* is the number of fish sampled at each location). Bold entries for latitude and longitude are weighted averages for multiple hauls in one geographic area and represent the sample sizes and coordinates used for each of the eleven "populations".

Sample no.	Sample name	Year	n	Latitude	Longitude
1	Queen Charlotte Island (QCI)	1999	46	54.460	-133.480
			2	54.660	-132.970
			2	54.630	-132.870
	combined totals		**50**	**54.475**	**-133.435**
2	Cross Sound (CSS)	2005	**100**	**58.205**	**-137.134**
3	Yakutat (YAK)	2005	**100**	**58.775**	**-140.426**
4	Cordova (COR)	1999	**100**	**59.270**	**-146.350**
5	Kodiak (KOD)	1999	**49**	**56.100**	**-153.500**
6	Shumagins (SHU)	2005	**100**	**54.677**	**-158.365**
7	Akutan (AKU)	1999	18	53.060	-166.530
			6	53.200	-166.200
			21	54.000	-165.330
			2	53.300	-165.300
			7	53.850	-163.890
	combined totals		**54**	**53.552**	**-165.639**
8	Central Aleutians (ALE)	2000	50	51.860	-174.170
			50	52.210	-172.950
	combined totals		**100**	**52.035**	**-173.560**
9	West Aleutians (WAL)	2000	**50**	**52.380**	**-179.690**
10	Southern Bering Sea (SBS)	2000	48	55.020	-167.300
		2000	51	55.410	-168.450
		2003	50	56.100	-168.440
		2003	50	56.020	-168.210
	combined totals		**199**	**55.638**	**-168.100**
11	Central Bering Sea (CBS)	2002	50	58.620	-174.660
			50	57.790	-174.190
	combined totals		**100**	**58.205**	**-174.425**
	GRAND TOTAL		1002		

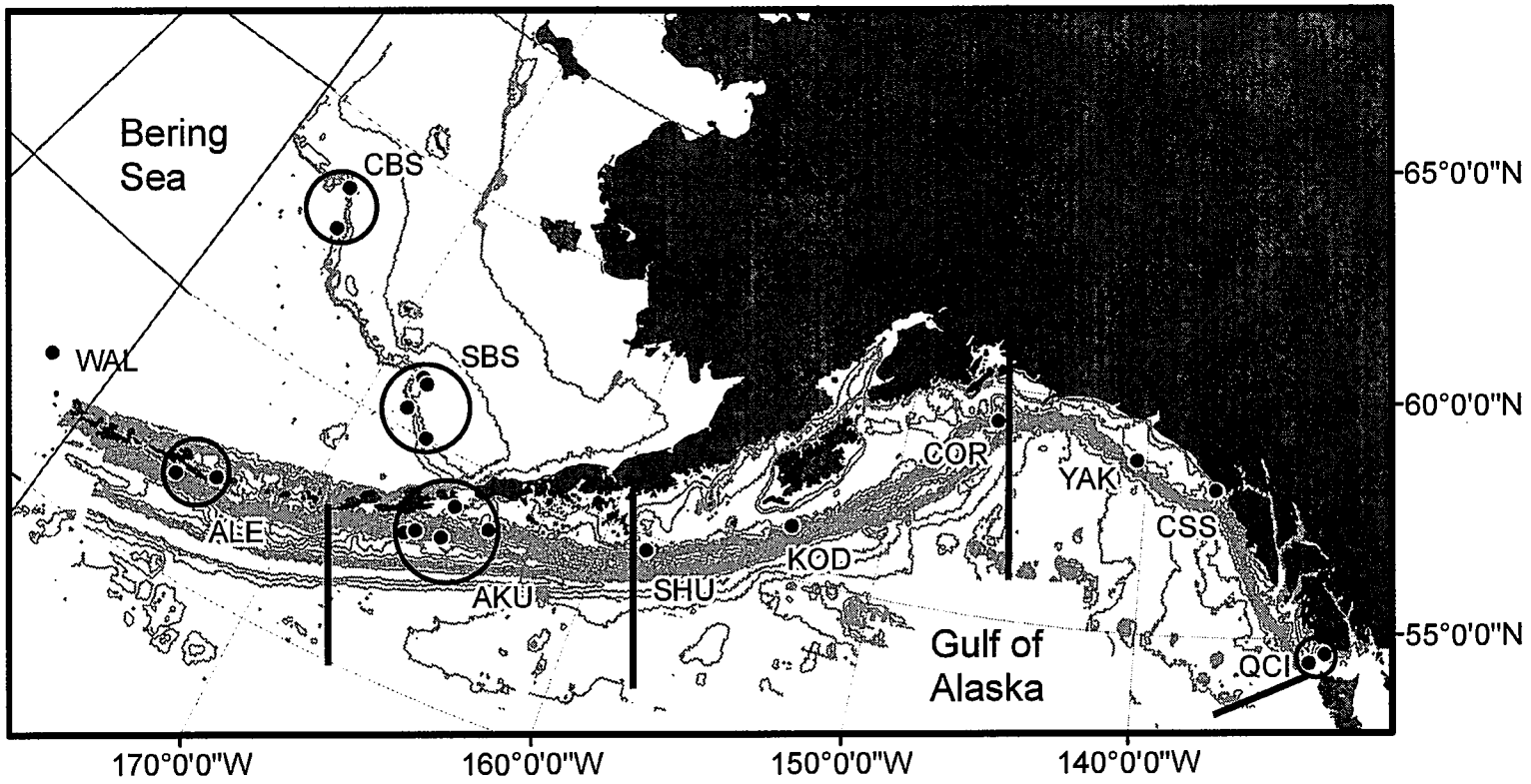

Figure 1. Map of collection sites and geographic groupings.

Geographic groupings are: Queen Charlotte Islands (QCI), Cross Sound (CSS), Yakutat (YAK), Cordova (COR), Kodiak (KOD), Shumagins (SHU), Akutan (AKU), Central Aleutians (ALE), Western Aleutians (WAL), Southern Bering Sea (SBS), and Central Bering Sea (CBS). Solid black lines represent management areas.

Microsatellite Amplification

After thirty microsatellite loci were tested for suitability, preliminary surveys were conducted with 17 loci: μSal1, μSal2, μSal3, μSal4, μSal6 (Miller et al. 2000); μSR7-2, μSR7-25, μSR7-7 (Westerman et al. 2005); μSma7, μSma5, and μSma11 (Wimberger et al. 1999); μSeb33 (Roques et al. 1999); μSpi4, μSpi6, μSpi10, μSpi12 (Gomez-Uchida et al. 2003); and μSth3B (Sekino et al. 2000). Three loci (μSma11, μSeb33, and μSr7-25) were removed from the analysis because they appeared to have abundant null alleles; fourteen microsatellite loci were used for the subsequent survey. Protocols differed for specific loci; but all were 10 μl reactions that included approximately 1 unit of Taq polymerase and final concentrations of: 1X PCR buffer (50mM KCl_2, 10mM Tris-HCl pH 9.0, 0.1% Triton x 100; Promega™, Madison, WI), 0.5 μM deoxyribonucleotide triphosphates, and approximately 0.05 to 0.10 μg DNA template; MgCl, primer, and DMSO concentrations were adjusted to optimize the reactions (Table A-1). Reactions included primers labeled with one of two IRDye® infrared dyes (LI-COR, Lincoln, Nebraska), which fluoresce at either 700 or 800 nm, to visualize the PCR products. The amplification profile was: 95°C for 5 min; 25-35 cycles of 0.5-0.75 min at 95°C, 0.5-0.75 min at annealing temperatures that ranged from 46°C to 60°C (Table A-1), and 0.5-0.75 min at 72°C; and 72°C for 3 min.

Microsatellite Analysis

Following PCR-amplification, 0.4 to 1.0μl of each sample and a LI-COR DNA standard (50-300bp) were loaded onto either a 0.25 or 0.4mm, 6% polyacrylamide gel (19:1 acrylamide/bisacrylamide) and run in 1X TBE (0.09M Tris-Borate, 2mM EDTA,

pH 8.3) buffer. Fluorescently labeled PCR products were visualized by using either a LI-COR LongReadIR™ 4200 or a LI-COR 4300 DNA sequencing system. Gels were run at 1500V for 1.5 to 2.0 hours, depending on the size of DNA fragments. Images of the gels were analyzed with the allele scoring program SAGA™ v3.2 (LI-COR, Lincoln, Nebraska).

Data Analysis

Allele frequencies were estimated with the software MSA (Dieringer and Schlötterer 2003) and GENEPOP v.3.2 (Raymond and Rousset 1995). Tests of conformance to Hardy-Weinberg frequency expectations (HWE) were performed for all combinations of loci and populations and evaluated with a pseudo-exact test in GENEPOP v.3.2. Tests of linkage disequilibrium between the locus pairs were performed with GENETIX v. 4.03 (Belkhir et al. 2004). A *G*-test, for which significance was evaluated by using Monte Carlo simulations, was also performed with this software to test for pairwise divergence between collections. Multiple testing was corrected with a sequential Bonferroni adjustment that had an overall α of 0.05 (Rice 1989).

The data were examined for correlations between genetic and geographic distance by an isolation-by-distance (IBD) model (Wright 1943, Mantel 1967). Geographic distances between collections were great circle distances along the continental shelf, with paths that circumscribed land masses. Pairwise (between collections) and individual F_{ST} (θ; Weir and Cockerham 1984) statistics were estimated with GENEPOP v.3.2, and plotted as $F_{ST}/(1-F_{ST})$ (Rousset 1997) against the corresponding geographic distance (km) to determine if there was a linear relationship. The same method was used to determine

the relationship when populations were separated into eastern and western GOA, and an ANCOVA analysis was used to compare the slopes of these two groupings. Isolation-by-distance analysis was also performed with geographic distance and genotype likelihood ratios (D_{LR}, Paetkau et al. 1997) as suggested in (Castric and Bernatchez 2004).

Genetic divergence was also used to examine the similarities between collections by calculating genetic distances. Trees were constructed from chord distances (Cavalli-Sforza and Edwards 1967), which were estimated with the computer program PHYLIP v.3.5 (Felsenstein 1993) with the CONTML program (Felsenstein 1993), a restricted maximum-likelihood method. Unrooted neighbor-joining trees (Saitou and Nei 1987) were also constructed to compare topologies. Treeview v.1.6.6 (Page 1996) was used to graph these relationships. Principal components analyses included alleles that had frequencies greater than 0.07 in at least one population. This approach reduced the influence of low-frequency alleles but incorporated most of the variation observed among populations. Allelic frequencies for each population were arcsine-square-root transformed and analyzed using SYSTAT v.11 (Systat Software Inc., Richmond, California) to obtain loadings of the first five components. The sum of the products of the component loadings and the arcsine-square root-transformed allele frequencies were used to plot the principal components for each geographic group.

I tested for correlations between allele frequencies and sample location to identify the primary allelic contributors to the divergence among collections. Tests were performed to estimate the correlations of the allele frequency with sample location by using both numeric order (east to west) and geographic (great circle) distances. Alleles

that were highly correlated with geographic location and had an average frequency of at least 10% over all collections were further analyzed by regression analysis with SYSTAT v.11.

Clustering algorithms, which identify groups of genetically distinct genotypes within the entire sample, can also be used to detect subgroup structure. The programs STRUCTURE v.2.1 (Pritchard et al. 2000, Falush et al. 2003), BAPS v.4.13 (Corander et al. 2003), and HWLER (Pella and Masuda 2001, 2006) estimate the number (K) of clusters (subpopulations) within a sample. All three methods were used to estimate K from the pool of all eleven populations.

Another approach to understanding population structure assigns individuals to the set of 11 populations that were defined. This approach examines the robustness and divergence of the adult POP genetic data and is often applied for stock identification. I used CBayes v.2.5 (Neaves et al. 2005) and SPAM v.3.7 (Debevec et al. 2000, Alaska Department of Fish and Game 2003), which have Bayesian resampling options. In both assignment methods, individual fish were reassigned back to the entire set of populations. I also used SPAM v3.7 to assign simulated mixtures of the populations to the entire data set. Assignments tests were also performed with GeneClass2 v.2.0.g (Piry et al. 2004). In these analyses, the individual that was being assigned was excluded (a leave-one-out procedure) from the estimates of allele frequencies of the populations.

Understanding the geneflow pattern or connectivity among these genetically distinct populations is an important component of describing the population dynamics and connectivity within a species and, ultimately, provides guidance for effective

conservation and management. Gene flow was estimated with MIGRATE v.2.2.2 (Beerli and Felsenstein 1999) and BAYESASSv.1.3 (Wilson and Rannala 2003). The program MIGRATE v.2.2.2. uses a maximum-likelihood method to determine the effective number of migrants ($4N_em$) by multiplying estimates of Θ and M ($\Theta = 4N_e\mu$, $M = m/\mu$), while BAYESASSv.1.3 uses a Bayesian framework. Connectivity was also estimated with CBayes v2.5 by assigning individuals to the baseline from which the collection of origin was excluded in order to observe into which other collection(s) they would be included. This latter approach provides information about the inter-connectivity between the geographic groups; but it does not measure directional movement between them.

Historical catch records show that Alaskan POP were fished to very low levels in the 1970s. Because this decline might have left a signal in the genetic data, I tested for evidence of recent severe demographic changes with the programs BOTTLENECK v.1.2.02 (Cornuet and Luikart 1996) and M_P_Val.exe (Garza and Williamson 2001). When a population passes through a bottleneck, rare alleles are lost faster than heterozygosity, which creates a theoretical "heterozygosity excess" relative to expectations from the perspective of a mutation-drift equilibrium that is based on the distribution of the number of alleles observed (Luikart and Cornuet 1998). Testing for significant "heterozygosity excess" in a population involves comparing the observed heterozygosity with the heterozygosity expected in a mutation-drift equilibrium population that has the same number of alleles under a particular mutation model. Two fundamental mutation models are the stepwise mutation (SMM) and the infinite allele (IAM) models. The SMM model is more restrictive than the IAM model because

mutations generally occur one "step" (e.g., one microsatellite repeat) at a time, as compared to the IAM model in which an allele can change from any state (allele) into any other state (allele). The infinite allele model (IAM), the stepwise mutation model (SMM), and several two-phase models (TPM), which combine the IAM and SMM models, were used and tested for significance with a Wilcoxon ranked sign test (Wilcoxon 1945, Wilcoxon and Wilcox 1964). Since microsatellites mutate primarily under the SMM model I examined combinations with greater than 70% SMM in the TPM (Shriver et al. 1993, Valdes et al. 1993). In addition, I used a range of mutation models to calculate Garza's M values for each population, which were compared to a null distribution for the same mutation model. The value M is the mean ratio of the number of microsatellite alleles to the range in allele size and should decrease following a reduction in population size because the rare alleles lost in bottlenecks are not always on the tails of the allele size distribution.

The effective population size (N_e) of each population was estimated to obtain an idea of local population sizes because that information may be valuable for future fine-scale management and provide additional information about connectivity. The approaches used by the programs included linkage disequilibrium, heterozygosity excess, and a Bayesian method that is currently under development (Hill 1981, Campton 1987, Bartley et al. 1992, Peel et al. 2004, Tallmon et al. in press). The program N_eEstimator (Peel et al. 2004) was used to estimate effective population sizes based on (1) the linkage disequilibrium method, which uses linkage distance between loci to determine the effective number of breeders, and (2) the heterozygosity excess method, which applies

the same theory as in the bottleneck estimates to determine the number of breeders. The program LDN_e (Waples and Do, unpublished, NOAA Northwest Fisheries Science Center) which was also used, applies the linkage disequilibrium method and corrects for bias in sampling size because estimates from samples smaller than the true N_e tend to be biased downward (England et al. 2006, Waples 2006). I used LDN_e to estimate N_e and compared the results to those of the linkage disequilibrium method that did not have a bias correction. An approximate Bayesian method was implemented in a program called OneSamp (Tallmon et al. in press) that uses summary statistics in a Bayesian framework to estimate N_e.

Another measure of population size that was applied to these data is an estimate of Wright's neighborhood size, N_b, which is defined as the number of individuals in the area from which parents of an individual can be drawn (Wright 1969). This is an appropriate estimate for the neighborhood model (Malecot 1975). The relationship between F_{ST} and the effective number of migrants (N_em), $N_e m = \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right)$, was used to provide a rough estimate of neighborhood size and effective population size by using the relationships between N_em and distance in Slatkin 1993 and between F_{ST} and distance described in Rousset 1997. In addition to linear distance (km), the number of steps between sample sites was also used to represent the space between neighborhoods. N_e was estimated from the y-intercept of the plots against linear distance and steps (Slatkin and Barton 1989, Slatkin 1993). The rationale is that the effective number of migrants (N_em) near 0 distance, where $m \rightarrow 1$, should be about N_e, which can be though of as a

neighborhood size (Slatkin and Maddison 1990). These estimates are biased by a term that is proportional to the constant A_1 described in Sawyer (1977) and Rousset (1997), the 1-dimensional plots in Rousset suggest that the influence may be small, but the value of the estimate is proportional to the reciprocal of the intercept, which is near 0, so it is difficult to judge its importance.

Dispersal distance, σ, can be estimated from the inverse of the slope of the regression of genetic $[F_{st}/(1-F_{st})]$ on geographic distance in linear habitats if the scale at which genetic divergence occurs is larger than the width of the habitat (Rousset 1997, Buonaccorsi et al. 2005). The inverse of the slope in this estimate is proportional to $4D\sigma^2$, where D refers to the density of individuals in the habitat and σ^2 refers to the variance of parental position relative to offspring position (Rousset 1997, Buonaccorsi et al. 2004, 2005). The program GENEPOP was used to estimate the slope of this relationship at both a population-and individual-level. Estimates of adult densities were used to solve for σ in this relationship. From census estimates conducted by NOAA Fisheries in their stock assessment surveys, estimates of adult densities per linear km ranged from 100 to 200 individuals (per comm. NOAA Fisheries, TSMRI, ABL). This linear density of POP was difficult to estimate because the width of the distribution is not uniform throughout the GOA, and because POP are patchily distributed throughout their range. Therefore, I performed a sensitivity analysis to determine how σ varied if the *effective* density was a fraction (0.5, 0.1, 0.05) of the linear adult density.

Another measure of genetic structure is the relatedness between individuals within a population or sub-population (Queller and Goodnight 1989, Lynch and Ritland 1999,

Ritland 2000, 2005, Wang 2002). Investigation into the relatedness in POP samples was performed, although significant relatedness was not expected because the collections were composed of multiple age classes. The program Identix v1.1 (Belkhir et al. 2002) was used to estimate three measures of average pairwise relatedness: the Lynch and Ritland (1999) measure, a pairwise adaptation of the Queller and Goodnight (1989) measure, and the Identity (Belkhir et al. 2002) measure. In addition to permuting the average pairwise relatedness of each geographic group, the variance in pairwise relatedness was also permuted.

Results

Genetic Variation Within Samples

A total of 998 fish was genotyped at fourteen microsatellite loci. The loci had between 15 and 51 alleles; their expected heterozygosities ranged from 0.345 to 0.964 and averaged 0.800 (Table 2). Only six of the 168 locus-by-population combinations differed significantly from Hardy-Weinberg expectations, fewer than would be expected by random chance; and no tests were significant after sequential Bonferroni corrections for simultaneous testing. There was no evidence of linkage disequilibrium for any pair of loci within any population after a sequential Bonferroni correction.

Genetic Structure Among Samples

Allele frequency distributions at each locus differed significantly among populations ($p < 10^{-5}$ for all loci), which indicated that POP are not genetically

homogeneous throughout their Alaskan range. All pairwise tests of homogeneity between populations were significant after Bonferroni correction, which further demonstrated the divergence among populations. The overall F_{ST} (θ) was 0.0123 and was significant based on permutation analysis ($p < 10^{-5}$). The F_{ST}s for individual loci were significant, except for μSma5 (p=0.067; Table 2). Populations that were composed of multiple collections were tested for genetic homogeneity; QCI, AKU, and CBS showed no significant differences among groups. Significant differences were observed between collections within ALE at five loci ($p < 10^{-3}$ over all loci). Four separate hauls, two sampled in 2000 and two sampled in 2003, contributed to SBS, which was the only location where a between-year comparison was possible. Significant genetic divergence was observed both within a year (2000, $p = 0.0127$; 2003, $p = 10^{-4}$) and between years ($p = 10^{-4}$). Because both between-year and within-year collections differed significantly, these genetic differences were most likely caused by fine geographic structure rather than interannual differences.

In order to further examine the observed divergence, I applied a wide range of genetic programs to characterize the structure within Alaskan POP. According to recent reviews and simulations, most of the commonly used genetic software works reliably on data sets that have an overall F_{ST} of at least 0.05, but they may not work well if F_{ST}s are smaller (Manel et al. 2005, Hauser et al. 2006, Latch et al. 2006, Waples and Gaggiotti 2006). Because the F_{ST} was 0.0123, I used a variety of genetic programs to evaluate the population differentiation that was observed so that the chances of detecting and understanding various characteristics of the underlying structure were maximized.

Table 2. Summary of population and locus characteristics for Pacific ocean perch.

For each locus the number of alleles, the range in allele size, heterozygosity with Nei's correction (H_e), inbreeding coefficients (F_{IS}) and overall F_{ST} (** $p < 10^{-3}$, Weir and Cockerham 1984) are reported (population abbreviations are found in Table 1). At the loci significantly ($p > 0.05$) out of HWE, F_{IS}'s are denoted with*; no correction was made for multiple testing.

Collection	Locus μSal1	μSal2	μSal3	μSal4	μSma7	μSr7-7	μSr7-2	μSma5	μSpi10	μSth3B	μSpi4	μSpi12	μSpi6	μSal6	All loci
QCI															
num. alleles	22	11	15	10	21	25	33	5	10	13	13	8	13	7	14.71
range	107-191	81-133	91-161	95-143	118-166	184-248	155-227	108-116	107-143	144-170	154-222	75-107	111-167	130-148	
H_e, Nei	0.924	0.807	0.844	0.773	0.918	0.939	0.962	0.221	0.808	0.831	0.800	0.592	0.805	0.692	0.780
F_{IS}, Pop	-0.051	-0.130	0.039	-0.041	0.121	0.009	0.033	0.014	0.094	0.000	0.010	-0.173	0.066	0.142	0.017
CSS															
num. alleles	20	11	13	15	23	27	37	9	14	16	15	9	15	8	16.57
range	107-207	85-125	96-166	83-147	94-164	180-246	149-229	100-152	95-163	138-170	154-226	75-109	103-159	132-148	
H_e, Nei	0.918	0.808	0.843	0.818	0.898	0.899	0.964	0.426	0.814	0.823	0.872	0.536	0.804	0.660	0.792
F_{IS}, Pop	0.055	0.014	-0.050	0.076	0.005	0.037	0.040	-0.098	-0.026	-0.065	-0.050	-0.021	0.010	-0.006	0.005
YAK															
num. alleles	21	14	13	15	23	26	37	5	10	15	18	8	17	11	16.64
range	103-191	81-141	91-161	83-143	118-164	188-246	155-241	108-116	107-147	142-170	154-230	75-107	103-171	118-148	
H_e, Nei	0.899	0.837	0.846	0.777	0.921	0.910	0.958	0.333	0.801	0.834	0.864	0.582	0.814	0.717	0.792
F_{IS}, Pop	0.016	-0.071	-0.071	-0.167	0.074	0.071	0.045	-0.075	0.027	0.082	0.010	-0.164	0.010	-0.009	0.001
COR															
num. alleles	24	14	12	14	26	25	42	12	9	12	18	12	15	11	17.57
range	103-207	81-149	86-151	83-147	104-166	182-246	147-247	102-128	107-167	144-166	154-230	73-107	103-159	128-148	
H_e, Nei	0.888	0.682	0.865	0.854	0.906	0.925	0.954	0.451	0.830	0.836	0.820	0.695	0.879	0.771	0.811
F_{IS}, Pop	-0.008	-0.017	-0.018	-0.107	*0.011	0.004	*0.043	-0.022	*0.002	0.057	0.036	-0.048	0.091	-0.007	0.012
KOD															
num. alleles	22	11	10	15	16	19	28	8	9	10	16	12	11	9	14.00
range	107-219	85-145	91-151	83-139	120-164	190-226	155-223	98-116	107-139	146-164	158-234	75-109	107-151	128-144	
H_e, Nei	0.911	0.641	0.806	0.861	0.887	0.920	0.944	0.387	0.792	0.839	0.786	0.723	0.805	0.778	0.791
F_{IS}, Pop	0.047	-0.072	-0.003	0.038	0.044	-0.010	0.037	0.009	0.005	0.062	0.050	-0.006	-0.004	-0.118	0.015

Table 2 (continued).

Collection	µSal1	µSal2	µSal3	µSal4	µSma7	µSr7-7	µSr7-2	µSma5	µSpi10	µSth3B	µSpi4	µSpi12	µSpi6	µSal6	All loci
SHU															
num. alleles	22	14	13	11	23	27	40	8	10	12	15	13	15	8	16.50
range	107-195	81-137	91-156	91-131	120-166	184-246	139-243	102-130	103-143	144-166	150-234	75-115	103-167	130-144	
H_e, Nei	0.856	0.687	0.830	0.850	0.898	0.913	0.947	0.334	0.818	0.868	0.735	0.752	0.880	0.802	0.798
F_{IS}, Pop	0.082	-0.072	-0.007	-0.054	0.002	0.019	0.044	-0.073	0.015	0.060	0.025	-0.070	-0.007	0.083	0.014
AKU															
num. alleles	24	11	9	12	20	24	33	4	9	13	15	12	14	9	14.93
range	107-211	81-125	106-161	91-143	118-160	190-242	145-227	110-116	99-147	144-172	158-222	75-107	107-159	126-146	
H_e, Nei	0.920	0.694	0.779	0.838	0.899	0.938	0.953	0.283	0.783	0.844	0.733	0.759	0.868	0.801	0.792
F_{IS}, Pop	-0.011	-0.085	-0.094	-0.064	-0.013	-0.013	0.003	-0.052	-0.037	0.043	0.120	0.062	-0.024	0.086	0.003
ALE															
num. alleles	24	12	12	14	25	28	35	7	9	14	17	13	16	9	16.79
range	107-215	81-125	91-146	83-139	102-166	182-242	143-225	108-146	107-139	136-170	154-238	75-109	103-167	130-146	
H_e, Nei	0.917	0.657	0.816	0.858	0.916	0.940	0.948	0.246	0.774	0.841	0.791	0.751	0.827	0.831	0.794
F_{IS}, Pop	0.012	0.001	0.024	-0.044	-0.010	-0.027	0.045	0.072	0.036	0.032	0.150	0.047	0.074	0.007	0.029
WAL															
num. alleles	21	10	8	12	24	24	31	5	9	10	15	12	12	9	14.43
range	107-215	81-121	106-141	83-131	118-164	176-242	139-227	108-116	107-143	146-166	158-234	75-111	111-155	128-148	
H_e, Nei	0.916	0.468	0.816	0.849	0.898	0.928	0.950	0.341	0.762	0.847	0.789	0.758	0.881	0.798	0.786
F_{IS}, Pop	-0.038	-0.016	-0.117	-0.051	0.119	-0.003	0.084	-0.045	0.066	*-0.053	*0.047	-0.018	0.057	-0.093	0.003
SBS															
num. alleles	29	13	14	14	25	30	43	12	15	14	19	13	16	10	19.07
range	107-219	81-129	81-171	83-151	106-164	182-248	145-243	100-184	99-159	138-172	150-230	71-109	103-167	128-146	
H_e, Nei	0.913	0.625	0.822	0.850	0.904	0.919	0.956	0.364	0.766	0.835	0.769	0.736	0.874	0.807	0.796
F_{IS}, Pop	0.001	-0.083	-0.017	-0.026	0.046	0.007	0.014	-0.018	-0.006	0.101	0.049	-0.022	-0.025	-0.038	0.002
CBS															
num. alleles	23	12	15	15	26	24	33	7	8	13	17	14	15	11	16.64
range	107-215	81-125	96-171	87-151	114-166	184-234	149-223	108-122	107-135	142-166	150-230	75-109	107-167	122-146	
H_e, Nei	0.890	0.702	0.855	0.861	0.934	0.930	0.944	0.288	0.793	0.817	0.836	0.686	0.876	0.825	0.802
F_{IS}, Pop	-0.018	-0.074	-0.048	0.006	0.010	0.016	0.020	-0.073	-0.129	0.102	0.062	0.003	-0.034	0.084	0.004
Summary overall populations															
num. alleles	30	18	19	18	34	36	51	20	19	18	23	20	18	15	15.90
range	103-219	81-149	81-171	83-151	94-166	176-248	139-247	98-184	95-147	136-172	150-238	71-115	99-171	118-148	
H_e, Nei	0.914	0.721	0.845	0.850	0.923	0.933	0.964	0.345	0.805	0.848	0.818	0.698	0.861	0.793	0.800
F_{IS}, Loci	0.010	-0.054	-0.029	-0.039	0.032	0.012	0.034	-0.038	-0.002	0.049	0.042	-0.032	0.011	0.009	0.004
F_{ST}, Loci	0.009**	0.039**	0.012**	0.010**	0.012**	0.008**	0.007**	0.005	0.011**	0.009**	0.017**	0.009**	0.008**	0.019**	0.012**

The analysis also provided an evaluation of the effectiveness of these methods for data that have relatively low F_{ST}s.

A test for correlation between genetic divergence, ($F_{ST}/(1-F_{ST})$, Rousset 1997), and geographic distance (km, shelf distance) indicated significant IBD ($R^2 = 0.505$, $p < 0.001$; Figure 2). The correlation between the genotype likelihood ratios (D_{LR}, Paetkau et al. 1997) and geographic distance (Castric and Bernatchez 2004) also had a significant, but weaker relationship ($p < 10^{-4}$, $R^2 = 0.25$; Figure A-1). These correlations established a geographic basis for the genetic divergence. To determine the allelic sources of this divergence in GOA populations, I tested correlations between the frequencies of abundant alleles (≥ 0.10 average frequency overall populations) and geographic distances between populations. Ten abundant alleles, which represented seven of the fourteen loci, had significant correlations ($p < 0.05$) with distance (km) from the eastern GOA to the western GOA (Figure 3).

The relationship among POP populations was also examined with a maximum likelihood tree (Figure 4). A neighbor joining tree of chord distances had a similar topology (not shown). The predominant geographic pattern observed in these analyses was a break between the eastern GOA (QCI, CSS, YAK) and the western populations. Each node in the tree was significant based on homogeneity tests ($p < 0.001$); but the western GOA populations and the Aleutian Island/Bering Sea populations appeared to be more similar to each other than to the eastern GOA populations.

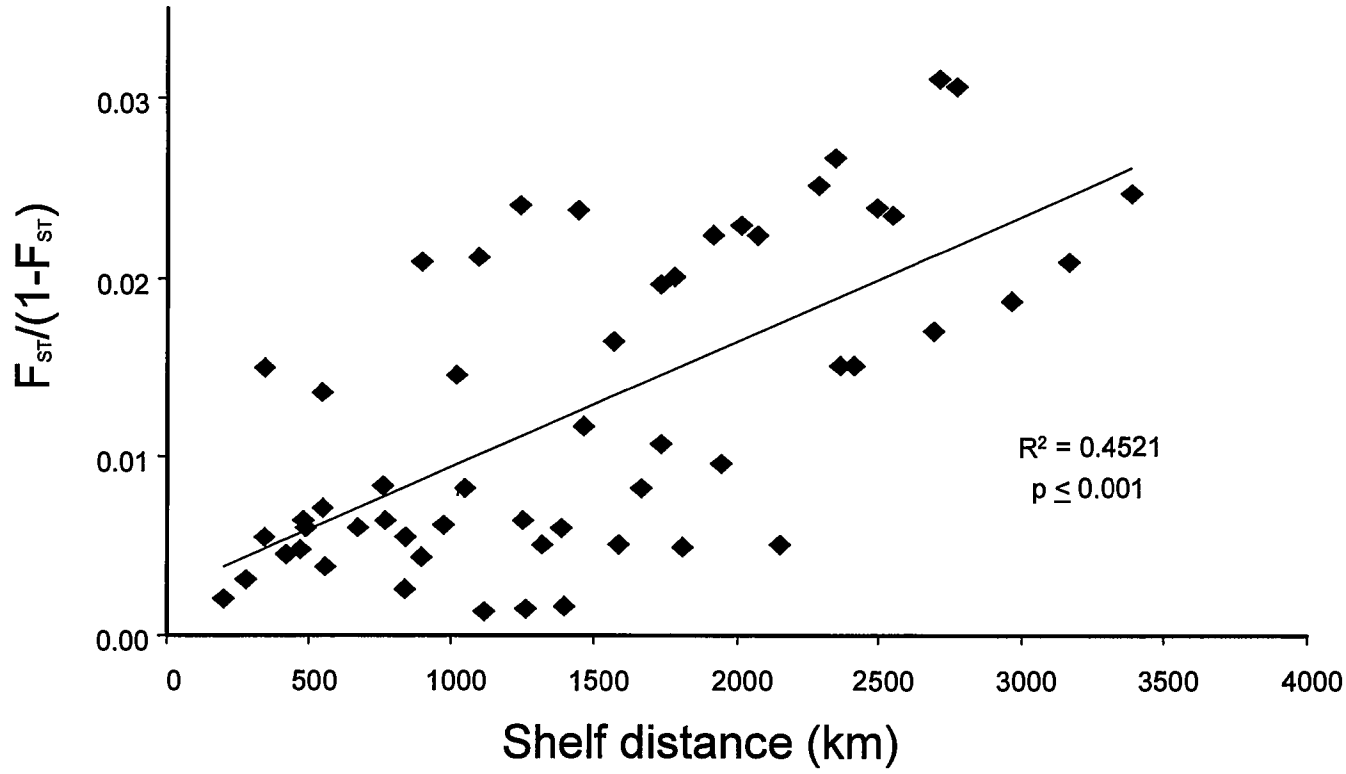

Figure 2. Isolation-by-distance of genetic (F_{ST}) and geographic (km) distances.

Genetic distance, represented as standardized pairwise F_{ST}s ($F_{ST}/(1- F_{ST})$), regressed on geographic shelf distance (km) ($y = 7.034 \times 10^{-6} x + 0.0024$).

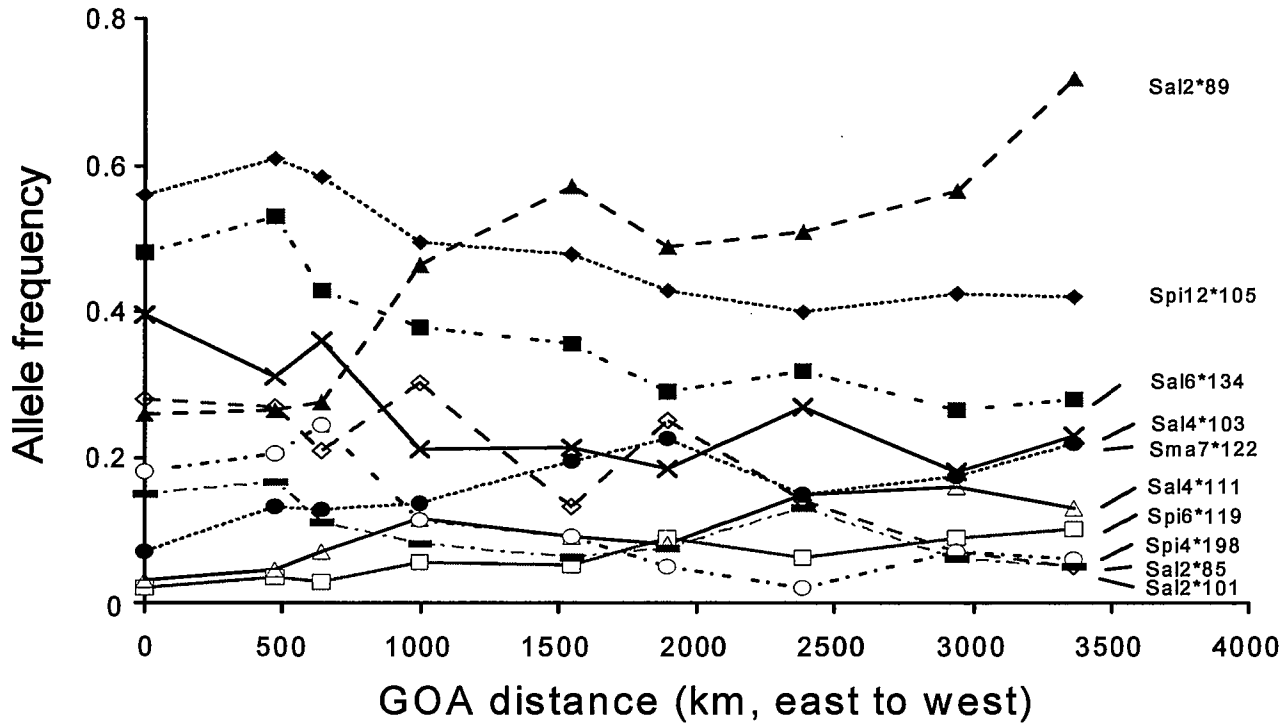

Figure 3. Allele frequency clines for POP in the Gulf of Alaska.

Plots of individual allele frequencies that had significant correlations with geographic distance from the eastern (QCI=0km) to the western (WAL=3400km) Gulf of Alaska. All regressions of allele frequencies against distance were significant from zero ($p<0.05$). μSal6*134 – $p<0.001$, μSal2*89, μSpi12*105, μSpi6*119, μSal2*101 – $p<0.01$, μSal4*103, μSma7*122, μSal4*111, μSpi4*198, μSal2*85 – $p<0.05$.

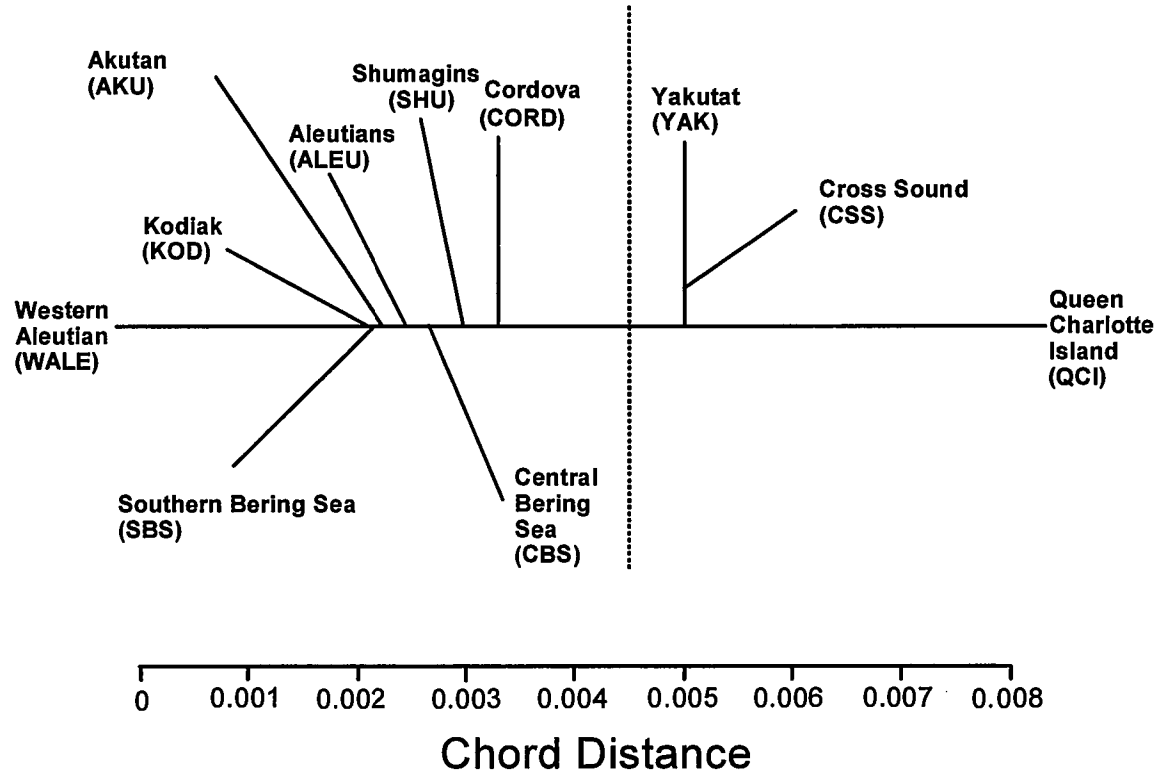

Figure 4. Tree of genetic distances among Alaskan Pacific ocean perch populations.

Maximum-likelihood tree from Cavalli-Sforza chord distances between geographic groups (CONTML in PHYLIPv3.5; Felsenstein 1993). Divergence among populations joined at each node is significantly different based on homogeneity tests. The vertical dotted line shows separation between eastern and western populations.

A principal components analysis (PCA) that used alleles with frequencies exceeding 0.07 also separated the eastern GOA from the other populations (Figure 5).

This geographic pattern prompted a reexamination of the isolation by distance analysis. Populations were separated based on the information from the genetic tree and PCA which divided them into eastern (QCI, CSS, YAK) and western (COR, KOD, SHU, AKU, ALE, WAL, SEB, SBS, CBS) groups. The regressions of geographic distances and pairwise genetic divergences, $F_{st}/(1-F_{st})$, performed within and between the two groups had parallel slopes for each group (ANCOVA, $p = 0.584$), but the intercept of the between-groups regression exceeded the within-group intercept (0.0157 ($F_{ST}/(1-F_{ST})$) compared to 0.0033 ($F_{ST}/(1-F_{ST})$); Figure A-2). This shows no difference in the IBD relationship, but suggests a greater genetic divergence between groups than within groups, which is to be expected. The programs STRUCTURE, BAPS, and HWLER are designed to detect substructure within an aggregation of individuals which do not have pre-defined groupings. The program BAPS was the only program that detected structure in this data set; it identified two clusters that were the same as those observed in the genetic tree and PCA analyses:

Program	**Estimation of K**
BAPS	2
	Cluster 1: {QCI, CSS,YAK}
	Cluster 2: {All others}
Structure	1
HWLER	1

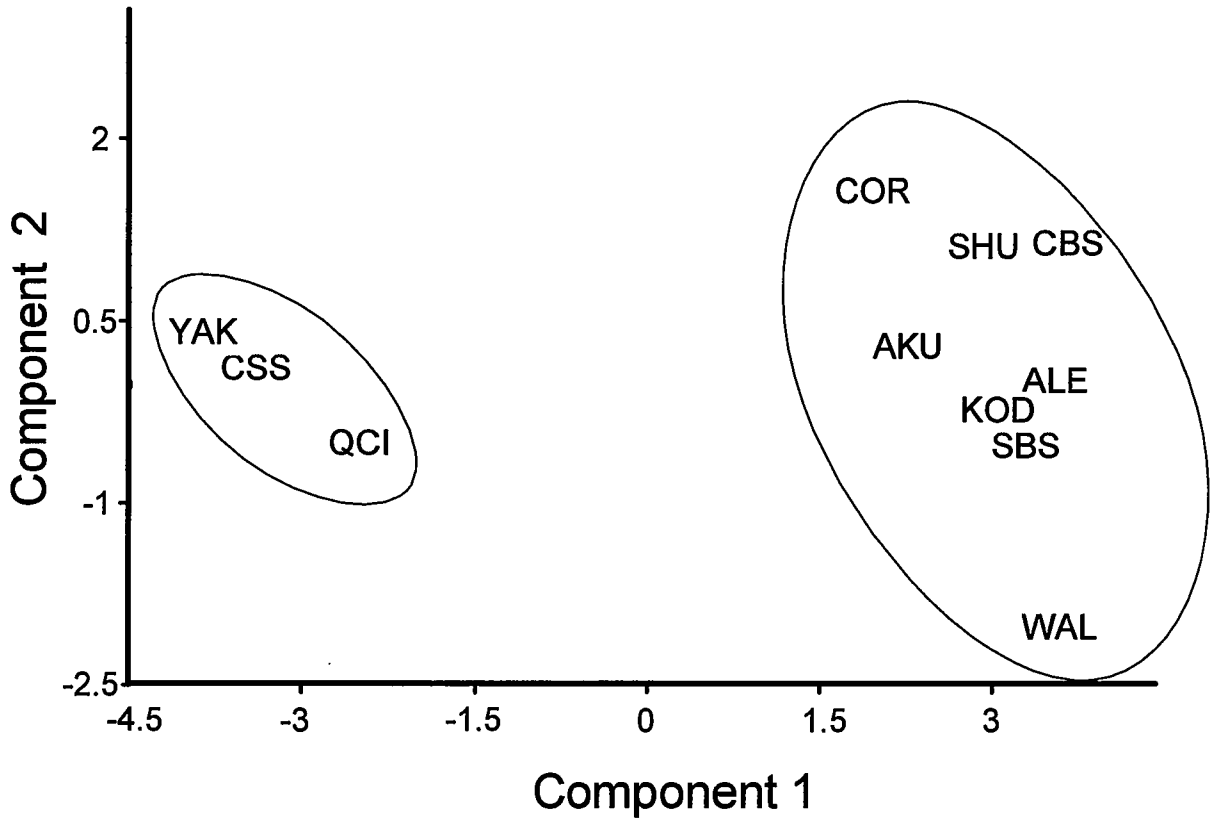

Figure 5. Principal component analysis of allele frequencies for Alaskan Pacific ocean perch populations. PCA of arcsine-square root transformed allele frequencies. Circles represent the two geographically separated clusters of populations.

Connectivity

Estimates of migration and gene flow can give insight into the connectivity of these populations. I used the programs MIGRATE and BAYEASS+ to investigate the east-west break between populations that occurred in the mid GOA by looking for connectivity indicated by estimates of geneflow. I did not obtain robust, meaningful estimates of parameters with these programs. In MIGRATE, convergence was never reached; and in BAYESASS+, the parameters converged to the default values, which indicated an inability to estimate true parameters from the data.

Assignment tests were also used to examine connectivity. These tests were conducted using CBayes v2.5. Individuals were assigned to the baseline one population at a time. Genotypic data for the population that was being assigned was omitted from the baseline, which forced assignment to a population other than the source. Based on the IBD analysis, it was expected that individuals would be assigned to the populations that were geographically close (Table 3). Some populations followed those expectations; and for QCI, CSS, YAK, SHU, WAL, and SBS, more than half of the individuals were assigned to adjacent populations. However, other populations COR, KOD, AKU, ALE, and CBS had assignment rates of more than 0.20 to geographically more distant populations (Table 3).

Table 3. Individual assignment probability for Alaskan Pacific ocean perch with the population of origin omitted. Assignment of individuals in which the population of origin was omitted from populations available for assignment with CBayes (Neaves et al. 2005). Individuals are assigned to population i (row) from population j (column). The proportion assigned to each population is presented along with its standard deviation (SD). Values larger than 0.20 are highlighted. Abbreviations for populations are the same as in Table 1.

	QCI	CSS	YAK	COR	KOD	SHU	AKU	ALE	WAL	SBS	CBS
QCI		0.034 (0.05)	0.052 (0.07)	0.003 (0.01)	0.006 (0.02)	0.026 (0.03)	0.005 (0.01)	0.006 (0.01)	0.005 (0.01)	0.001 (0.00)	0.006 (0.01)
CSS	**0.404** (0.14)		**0.928** (0.08)	0.120 (0.05)	0.003 (0.01)	0.004 (0.01)	0.024 (0.04)	0.001 (0.00)	0.003 (0.01)	0.001 (0.00)	0.002 (0.01)
YAK	**0.374** (0.14)	**0.933** (0.06)		0.065 (0.04)	0.004 (0.01)	0.004 (0.01)	0.058 (0.04)	0.001 (0.00)	0.002 (0.01)	0.001 (0.00)	0.002 (0.01)
COR	0.004 (0.01)	0.009 (0.02)	0.003 (0.01)		0.012 (0.03)	**0.253** (0.20)	0.004 (0.01)	0.004 (0.01)	0.006 (0.01)	0.021 (0.03)	0.056 (0.05)
KOD	0.006 (0.02)	0.002 (0.01)	0.003 (0.01)	0.025 (0.03)		**0.383** (0.26)	0.017 (0.03)	0.164 (0.14)	0.107 (0.08)	0.191 (0.07)	0.008 (0.02)
SHU	0.061 (0.04)	0.002 (0.01)	0.002 (0.01)	**0.422** (0.09)	0.062 (0.08)		0.006 (0.02)	0.027 (0.04)	0.003 (0.01)	0.016 (0.02)	0.069 (0.08)
AKU	0.041 (0.05)	0.002 (0.00)	0.001 (0.00)	0.013 (0.03)	0.009 (0.03)	0.004 (0.01)		0.039 (0.06)	0.026 (0.05)	0.050 (0.06)	0.008 (0.02)
ALE	0.034 (0.04)	0.002 (0.00)	0.002 (0.01)	0.007 (0.02)	0.153 (0.15)	0.015 (0.03)	0.054 (0.07)		0.005 (0.02)	**0.221** (0.07)	**0.812** (0.08)
WAL	0.004 (0.01)	0.002 (0.00)	0.001 (0.00)	0.002 (0.01)	0.041 (0.08)	0.006 (0.02)	0.021 (0.04)	0.032 (0.05)		**0.390** (0.07)	0.006 (0.02)
SBS	0.069 (0.05)	0.015 (0.02)	0.002 (0.00)	0.083 (0.06)	**0.639** (0.14)	0.181 (0.08)	**0.774** (0.09)	**0.509** (0.11)	**0.839** (0.10)		0.031 (0.05)
CBS	0.003 (0.01)	0.001 (0.00)	0.006 (0.01)	**0.260** (0.07)	0.070 (0.09)	0.124 (0.09)	0.036 (0.05)	**0.217** (0.12)	0.004 (0.01)	0.108 (0.05)	

Assignment tests conducted with CBayes v2.5 and SPAM v3.7 were also used to determine the robustness of this data set as a baseline for use in future studies of Alaskan POP population structure. In CBayes, each population was treated as a collection from a mixture; whereas in SPAM, a series of simulations were performed in which mixtures were sampled entirely from a single population. Self-assignment rates were high, greater than 0.75, for both methods (CBayes between 0.89 and 0.98, SPAM between 0.76 and 0.93; Figure A-3, Figure A-4); however, neither method has a leave-one-out feature in which the individual that is being assigned to the baseline is excluded from the baseline allele frequency estimation. Therefore, an additional program that performed individual jackknifing was used GeneClass2 v.2.0.g. With this method, the assignment rates decreased to between 0.10 and 0.48, but the population of origin still had the highest assignment rate for the majority of populations (Table A-2). These assignment rates are low in comparison to the range of assignments seen without jackknifing individuals in GeneClass2 (between 0.50 and 0.80). This is probably due to the presence of low frequency alleles, which were partially compensated for by using a Bayesian method to estimate allele frequencies in each population (Baudouin and Lebrun 2000). In these analyses, the prior distributions that were used relied mostly on the observed data, which had many low frequency (< 0.02) alleles. After the low frequency alleles (< 0.02 overall) were pooled together, I obtained higher self-assignment rates with the jackknifing method (between 0.15 and 0.60 compared to between 0.10 and 0.48); and the average difference in assignment between the non-jackknifing (mixture) and jackknifing methods decreased (0.25 with pooling, compared to 0.44 without pooling, Table A-3, Table A-4). Such low

self-assignment reflects the similarities that exist between the collections, which one would expect with low but significant variation, and the influence of low frequency alleles on assignment methods.

Bottleneck

Because there have been historic POP population declines, I tested for recent detectable genetic bottlenecks. Under the IAM model, the Wilcoxon (1945) ranked sign tests provided no evidence for a genetic bottleneck in any population, which is indicated by the lack of excess heterozygosity relative to that expected for the number of alleles observed. The two-sided Wilcoxon tests were significant for a heterozygosity deficit when the two-phase model included more than 70% of the stepwise mutation model (SMM). At 100% SMM, all populations had significant heterozygosity deficits ($p < 0.05$); at 90% SMM (10% IAM), six populations were significant (QCI, CSS, YAK, COR, AKU, SBS); and at 80% SMM (20% IAM), two populations were significant (CSS, YAK). When the model was at less than 70% SMM (30% IAM), no significance was observed in two-tailed Wilcoxon tests. Garza's M value provided no evidence for bottlenecks when compared to the null distribution that had the same number of alleles under multiple mutation models (Garza and Williamson 2001).

Effective Population Size

Estimates of effective population sizes, N_e, for each population were made with three methods. Both the heterozygosity excess and the Bayesian methods included infinity in estimates of N_es for all populations. In contrast, the linkage disequilibrium

method estimated non-infinite N_e point estimates for nine out of the twelve populations. Those estimates ranged from 170 to 7000, however, all but four of the upper confidence intervals on these estimates were infinite (Table 4). The program LDN_e (Waples and Do, unpublished, NOAA Northwest Fisheries Science Center), corrects for some of the bias involved with small sample size in comparison to a large N_e. This program gave similar estimates of N_e but also suggested infinite upper confidence intervals (Table 4). A robust estimate of N_e does not seem possible with these data, probably because the effective population sizes are large. It is difficult to distinguish a large N_e from an infinite N_e (pers. comm. Robin Waples) and this method also has high variability when the sample size is much less than the true N_e (Waples 2006).

Another estimate of the effective size of populations is an estimate of neighborhood size (Wright 1969). The relationships between N_em and distance in Slatkin (1993) and between F_{ST} and distance described in Rousset (1997) were used to estimate effective numbers of individuals and dispersal (Slatkin and Barton 1989). The following regressions resulted in the slopes and intercepts listed: Fst/(1-Fst) vs. distance(km) ($y = 6.68\times10^{-6}x + 0.005$), Fst/(1-Fst) vs ln[km] ($y = 0.0072x - 0.0365$), logNm vs km ($y = -2.6\times10^{-4}x + 1.7046$), log Nm vs. ln[km] ($y = -0.6663x + 3.3853$), and log Nm vs. steps ($y = -0.1199x + 1.7445$), where steps indicate the order sample sites, and are probably not demes. All of these regressions are significant, but this significance should be interpreted cautiously because the values regressed are correlated.

Table 4. Estimation of the effective population sizes of populations of Alaskan Pacific ocean perch.

Estimates were performed for each geographic group with the linkage disequilibrium method in N_e Estimator (Peel et al. 2004) and the program LDN_e (Waples and Do, unpublished, Northwest Fisheries Science Center). Included are the lower 95% confidence interval (L C.I.) and the upper 95% confidence interval (U C.I.). *Negative values in LDN_e are interpreted as infinite values.

	N_e (N_e Estimator)			N_e (LDN_e)		
	L C.I.	value	U C.I.	L C.I.	value	U C.I.
QCI	651	3314	infinite	791	*-2771	infinite
CSS	1301	4752	infinite	1790	*-3200	infinite
YAK	1391	5710	infinite	657	1604	infinite
COR	388	523	792	136	161	195.8
KOD	834	infinite	infinite	333	932	infinite
SPT	862	1644	14225	715	1910	infinite
SHU	287	449	994	369	1014	infinite
ALE	2239	infinite	infinite	663	1564	infinite
WAL	724	7481	infinite	1172	*-1138	infinite
SBS	10936	infinite	infinite	1774	7323	infinite
CBS	5644	infinite	infinite	726	1906	infinite

The estimates of N_em at 0 for all three of the Nm regressions were similar, ranging from 50.2 to 55.5 individuals. The slopes of Rousset's (1997) relationships, from both sample- and individual-level regressions, were significant (Mantel test in GENEPOP: sample, p=0.0004; individual, p<0.0001). The estimates of the slopes were similar, 6.61×10^{-6} to 6.68×10^{-6}. The inverses ranged from 149,607 and 151,297 individuals. These estimates were then used along with estimates of adult linear density (100-200 individuals per km) to estimate, σ, which is the standard deviation of dispersal for a range of *effective* adult densities (0.5, 0.1, and 0.05 of the linear adult density). *Effective* adult densities were used for a sensitivity analysis, to determine the influence of adult density on the estimate of dispersal. For the lower estimate of density (100), σ ranged from 20-90km, and for the higher estimate of density (200), σ ranged from 14-60km for the different *effective* densities used in the sensitivity analysis.

Relatedness

In a large population, average pairwise relatedness should be close to zero; significant relatedness will only be observed if few related families are involved in producing offspring, which would increase inbreeding and, consequently, relatedness within the population (Ritland 2005). Thus, estimating relatedness is another way to detect population substructure. Relatedness within POP populations was estimated with three different methods. Average pairwise relatedness was not significant for any population as estimated from permutation tests (Table 5a). For Akutan alone, the Queller and Goodnight (1989) method of estimating pairwise relatedness was significant (p = 0.047), but the two other methods were not. The amount of relatedness observed did not

exceed what would be expected in a panmictic population. The variance in average pairwise relatedness was also tested for significance with a permutation test. The variance was significantly ($p < 0.05$) higher than expected under a panmitic population for three populations for all three pairwise relatedness estimation methods (YAK, COR, ALE), for two with two methods (CSS, SHU), and three populations with one of the methods (QCI, KOD, AKU; Table 5b). The Bering Sea samples (SBS, CBS) did not have a significant variance in pairwise relatedness, even though SBS was composed of multiple collections; this may be because of the broad sampling scale. A variance of pairwise relatedness that is larger than expected suggests that the sample may be composed of many smaller related groups, which is consistent with finer geographic substructure within the populations defined in this study.

Table 5. (a) Pairwise relatedness and (b) Variance in pairwise relatedness for Alaskan Pacific ocean perch populations.

(a) Percent chance that the average pairwise relatedness would be lower than observed under a random distribution of the observed allele frequencies based on three alternative methods (Lynch & Ritland 1999, Queller & Goodnight 1989, and Identity). These values were calculated with the program Identix v1.1 after 1000 permutations of alleles; values less than 5% are significantly more related than expected with a random distribution.

	QCI	CSS	YAK	COR	KOD	SHU	AKU	ALE	WAL	SBS	CBS
Lynch and Ritland	48.50%	87.80%	85.40%	41.30%	N/A	39.10%	24.20%	73.00%	N/A	16.20%	26.00%
Queller and Goodnight	5.20%	98.70%	69.80%	32.90%	29.10%	40.70%	**4.70%**	46.90%	69.60%	50.10%	64.00%
Identity	98.10%	98.60%	98.90%	73.00%	86.10%	92.80%	42.20%	91.10%	76.30%	84.80%	78.10%

Mean after alleles permutated

(b) The percent chance that the variance in pairwise relatedness within geographic groups is larger than expected under a random distribution of the observed allele frequencies. The values were determined with the program Identix v1.1 and the three methods used above after 1000 permutations of each. Values less than 5% have a significantly higher variance than expected.

	QCI	CSS	YAK	COR	KOD	SHU	AKU	ALE	WAL	SBS	CBS
Lynch and Ritland	14.00%	**2.10%**	**0.30%**	**0.20%**	13.80%	**1.00%**	**4.20%**	**2.60%**	55.60%	31.50%	35.80%
Queller and Goodnight	**0.30%**	**0.20%**	**1.00%**	**1.00%**	5.20%	7.20%	16.40%	**1.00%**	37.60%	92.80%	20.20%
Identity	21.90%	7.90%	**0.90%**	**0.40%**	**0.60%**	**0.60%**	13.60%	**1.10%**	53.60%	67.60%	32.60%

Variance after alleles permutated

Discussion and Conclusions

Larvae of many marine fishes have the potential to disperse widely because they have extended planktonic life stages that are susceptible to transport by ocean currents (Roberts 1997, Bohonak 1999, Stepien 1999). In addition, adults of some species can swim great distances. This combination of larval and adult movements can result in panmixia (Gold et al. 1994, Palumbi 1994, Stepien 1999). In the GOA, the Alaska Gyre and other currents provide opportunities for transporting passive larvae, generally in a counter-clockwise pattern, to the northwest (Moser and Boehlert 1991, Hinckley et al. 2001). In spite of the potential for larval drift and adult movement, my observations of adult POP indicated strong genetic structure. Pairwise tests, homogeneity, IBD, and allele frequency clines were all consistent with strong divergence and geographically-based population structure in Alaskan POP.

Fine-scale genetic structure was previously reported in POP within a relatively small geographic area in British Columbia (Withler et al. 2001). However, that locale had strong geographic and oceanographic features, which have the ability to isolate different population segments. The genetic divergence that I observed was based on samples collected over a much wider geographic scale, and the physical oceanography along the Pacific rim does not have obvious features that might give rise to discrete isolates over much of the area that was sampled. The divergence I observed indicates that the geographic sampling scale was coarser than the apparent natural neighborhood/ population scale.

One aspect of the POP structure was a discontinuity between the eastern and western GOA populations on a Gulf-wide scale. This discontinuity coincides with a biogeographic break in groundfish diversity in the GOA that was described from NMFS groundfish survey data and occurs just south of Prince William Sound between Yakutat and Kodiak areas (Mueter and Norcross 2002). Similar, but weaker, patterns have also been observed for POP congeners, such as shortraker (*S. borealis*, Matala et al. 2004a) and rougheye rockfish (*S. aleutianus*, Gharrett et al. 2007). This break may be influenced by a combination of POP life history characteristics and biogeographic boundaries, which in turn may be influenced by oceanographic currents, historic distributions, or association with particular habitats. Specifically, the area between Yakutat and Cordova is oceanographically complex; in this area the narrow Alaska Coastal Current widens its influence as the shelf broadens and the formation of the strong Alaskan Stream begins to play a dominant role in shelf edge transport (Stabeno et al. 2004).

Little is known about the timing and location of POP parturition, therefore variations in them may contribute to the observed genetic discontinuity (Gray et al. 2006). POP larvae are released in the spring, when oceanographic changes occur in the GOA as the stormy winter season ends and the calm summer begins. Consequently, the timing and location of their release may determine the extents and paths of their dispersals. The retention of larval POP in mesoscale features, such as eddies that have been observed to form episodically in the eastern Gulf at various scales, could be a possible mechanism for a discontinuity in gene flow because some larvae may be retained in the eastern Gulf by these eddies, while others are caught up in currents and

transported westward along the shelf or seaward into the GOA basin where they may not successfully recruit back to adult populations. For example, the area between Yakutat and Cordova is oceanographically dynamic, characterized by seasonal eddy formations (Stabeno et al. 2004). Drifter trajectories have shown reoccurring eddy formation west of Kayak Island, which is to the east of the Cordova collection (Stabeno et al. 2004). This eddy may prevent eastern GOA larvae from moving further westward, and contribute to genetic discontinuities. The extent to which oceanographic patterns influence the dispersal of POP larvae needs to be studied further in order to better understand their population structure and connectivity.

Other influences on the genetic diversity of some long-lived marine species, such as POP, are sweepstakes effects and bottleneck events. Sweepstakes effects describe genetic drift that may occur among larval cohorts as a result of high variances in family size (Li and Hedgecock 1998, Flowers et al. 2002). While the influence of a sweepstakes effect cannot be ruled out, it is unlikely that it would produce the strong correlation between genetic divergence and geographic distance that was observed.

Bottleneck events, in which genetic diversity is reduced, occur when populations decline to severely low abundances. Populations of POP decreased dramatically from overfishing in the 1960-70s, approximately 30 years before these samples were taken; however, those declines do not seem to have been large enough to leave genetic signals that are detectable by currently available bottleneck analyses. Restoration of genetic divergence following bottleneck events involves accumulation of mutations and reduction of genetic drift. Geneflow can also restore variation. Mutation acts too slowly

to be of consequence, and my results indicate that geneflow probably had little influence in that time span, which leaves random drift. Pacific ocean perch are long-lived (100+ years) and late-maturing (50% maturity is approximately 10.5 years); if populations had declined to extremely low levels there would most likely be a detectable signal even after 30 years. Although a bottleneck event was not detectable, which means only that the population did not reach critically low levels from a genetics perspective, the decline in POP abundance could have influenced present day distributions and may have played a role in the population structure.

The characterization of structure and connectivity of POP populations was limited by the inability of many statistical estimators to perform well at low levels of divergence. Homogeneity tests, IBD, and PCA analyses recognized structure within POP, even at an overall F_{ST} of 0.0123. However, methods that estimate migration rates, such as MIGRATE and BAYESASS+, did not provide meaningful estimates, and model-based clustering methods that were used to estimate the number of contributing groups (K) performed poorly; only BAPS successfully identified the large-scale structure within Alaskan POP. Finally, assignment methods, such as CBayes and Geneclass, had decreased assignment rates when the individual that was being assigned was removed from the baseline. Overall, available genetic tools, even ones that used Bayesian approaches, did not adequately characterize population structure when low, but significant, divergence existed. In spite of these analytic limitations I was able to construct a coarse preliminary description of POP population structure.

The dispersal of just a single life history stage can result in a homogeneous population in marine fishes; consequently, for a population to be genetically structured, the movements of larvae, juveniles, and adults must all be limited geographically. Surviving larval fish may be locally retained near their area of release (short dispersal), they may have vertical migratory behavior that keeps them close to their natal area, or they may have directed movement back to their parturition site or natal population as juveniles or young adults (homing). Limited larval dispersal has been observed in other rockfish species and was hypothesized as the primary mechanism that contributed to the genetic divergence in some *Sebastes* species (Withler et al. 2001, Buonaccorsi et al. 2002, 2004, Miller and Shanks 2004).

Based on the genetic structure observed, the movement of adults must also be restricted and at least the spawning populations belong to relatively small (at my sampling scale) "neighborhoods" or localized populations. Because adult fish were not sampled from spawning populations, the observed structure cannot be explained simply by homing to spawning areas. It appears to be a more static structure. This implies that adult POP may associate with features in their habitat, as has been described for other *Sebastes* species (Scott 1995, Brodeur 2001, Mitamura et al. 2002, Johnson et al. 2003). Adult POP have been observed in schools that congregate demersally in benthic depressions or gullies along the continental shelf break (Brodeur 2001, Lunsford et al. 2001). The extent of movement of these schools is unknown, but annual depth migrations have been observed; adults inhabit shallow waters in the summer (200-275m) and move into deeper waters (300-450m) in the winter (Gunderson 1972, 1977, Scott

1995). Such behavior may be a component of their fine-scale population structure. Even though dispersal is limited, the strong IBD signal and overall northwestward movement of gene flow (Figure 3, Table 4) implies limited connectivity between neighborhoods that is accompanied by a low level of gene flow by larvae and/or adults.

Describing the demographics of these adult POP neighborhoods is constrained by the scale of sampling in this study, a lack of comprehensive life history information for POP, and the low but significant genetic differentiation ($F_{ST} = 0.0123$). Although population structure was apparent, the design of the sampling scale was too large (approximately 400 km between samples) to adequately characterize finer structure or to determine the scale of population structure. Both the divergence between collections within ALE and SBS and the significantly high variance in relatedness within populations provided evidence for structure that has a finer scale than the sampling scale. However, without more intensive sampling I was only able to roughly estimate the size of the neighborhoods or localized populations. The estimates of neighborhood size (N_b) were conflicting because they depend on the geographic scale. However, I could approximate N_e at 0 distance (50 individuals) and the dispersal distance, σ (14-90km), both of which suggest limited effective neighborhoods compared to actual adult biomass. As an indirect estimate of dispersal, these distances should be used with caution because they include errors from estimates of both genetic diversity and density. The lower range of the estimate of σ is based on estimated adult densities of 100-200 individuals/km and most likely underestimates the true dispersal distance. The larger values of σ were estimated from a range of plausible *effective* densities (Whitlock and McCauley 1999,

Buonaccorsi et al. 2005). Both dispersal and *effective* numbers estimates indicate that population structure may be more complex than simple localized populations. Although the N_e at 0 distance estimates were small, the estimated effective population sizes were uniformly large, which suggests that there is no measurable influence from the number of breeders at this sampling scale (England et al. 2006, Waples 2006).

The scale of population structure observed here was finer than the scale that is used to manage Alaskan POP. The POP management areas are divided into the GOA and Bering Sea/Aleutian Islands (BSAI); the GOA is further divided into eastern, central, and western management areas (Figure 1) and the BSAI is further divided into the Bering Sea and the Aleutian Islands management areas. In the GOA, each management area may encompass multiple populations, which could create the potential for over-harvesting of some populations and under-harvesting of others. Rockfish, which are long-lived species, are slow to recover from over-harvesting. Management at geographic scales that substantially exceed the scale of population structure may make those populations susceptible to overfishing and reduced productivity (Parker et al. 2000). In contrast to POP, the spatial scales of population structures of other rockfish species, such as shortraker (*S. borealis*) and rougheye (*S. aleutianus*), along the western coast of North America appear to be larger and more consistent with the spatial scales of boundaries used for management (Matala et al. 2004a, Gharrett et al. 2007). While these three species of rockfish inhabit the same areas, they differ in feeding behavior; POP are planktivorous, whereas rougheye and shortraker rockfish are carnivorous. In addition, POP are found in large aggregations while these other species do not appear to exhibit

this behavior, which may also influence their population structure. Determining an effective management scale for adult POP will involve characterizing their aggregations as either neighborhoods or localized populations, determining the geographic scale of these units, and estimating dispersal distance.

There are still many unanswered questions about POP life history that deserve attention. First, confirmation is needed to demonstrate that sweepstakes effects do not contribute substantially to genetic divergence; second, a more precise estimate of the scale of POP neighborhoods is needed. The scale of population structure depends on the extent of larval dispersal and the movement of juveniles and adults, which could be estimated with finer spatial scale sampling. Dispersal and neighborhood size of POP need to be evaluated in terms of the bathymetric and oceanographic features within their distribution range. Ultimately, a method that incorporates relevant life history information into a population model for POP will provide a basis for estimating the effects of spatial scale on the management of POP and other marine species. Although more research is necessary to complete the picture of POP life history, my results complement recent studies that suggest that broad larval dispersal and/or the presence of panmictic marine populations are not universal in marine fishes, particularly rockfishes (Bentzen et al. 1996, Rocha-Olivares and Vetter 1999, Gomez-Uchida and Banks 2005, Miller et al. 2005, Ruzzante et al. 2006). In conclusion, this coarse-scale genetic analysis contributed some insight into POP population structure and dispersal, but more research is needed to further illuminate the complex life history of Alaskan POP.

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Appendix

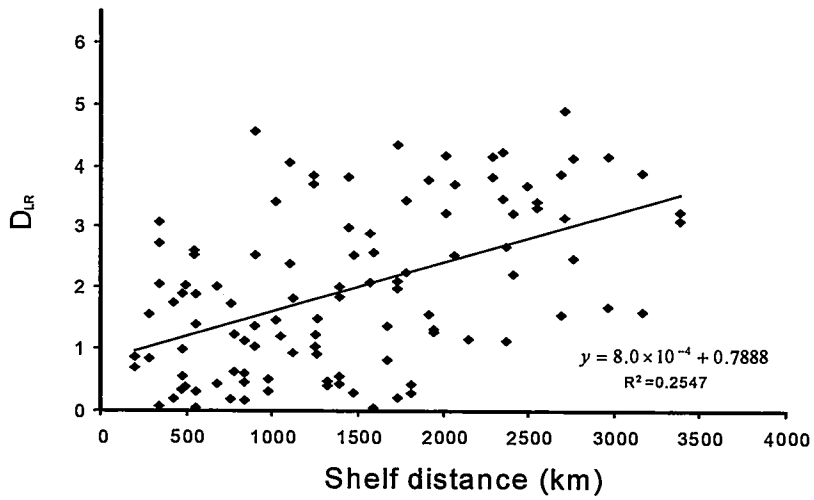

Figure A-1. Isolation-by-distance of geographic shelf distance (km) and genotype likelihood ratios (D_{LR})

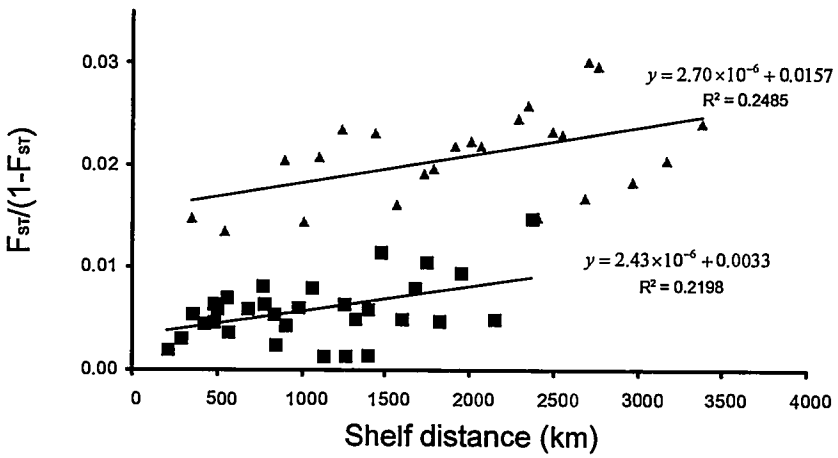

Figure A-2. Isolation-by-distance between eastern and western GOA.
Isolation by distance within and between two geographic groupings, east GOA (QCI, CSS, YAK) and the rest (COR, KOD, SHU, AKU, ALE, WAL, SEB, SBS, CBS). Squares are within group pairwise comparisons of $F_{st}/(1- F_{st})$ and triangles are between group pairwise comparisons. The slopes are parallel and not significantly different

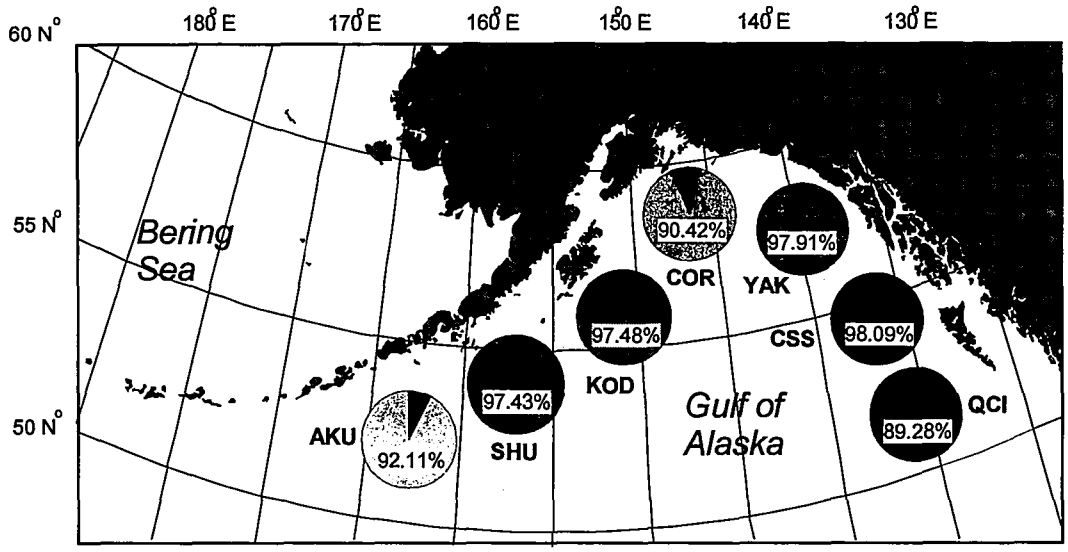

Figure A-3. Assignment rates using CBayes v.2.5.

Individual assignment rates to populations in the Gulf of Alaska (GOA) from CBayes; self-assignment rates are labeled on the pie charts.

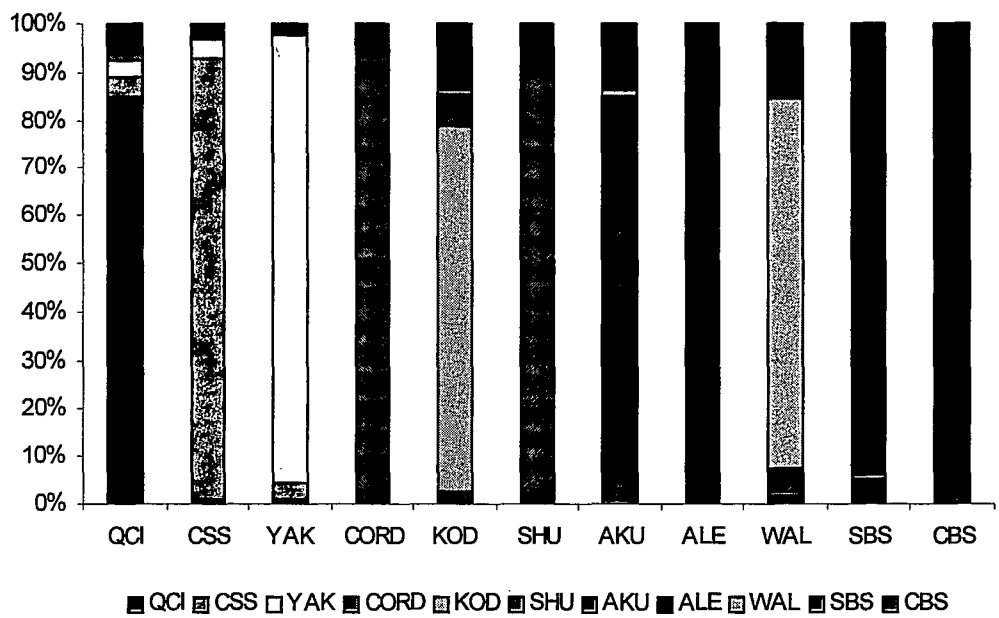

Figure A-4. Simulation assignment rates using SPAM.

Simulations using the program SPAM of one hundred percent from each geographic group.

Table A-1. Amplification reaction conditions.

All components are reported as final concentrations in a 10 µL reaction. Loci in italics were not included in the final analysis.

	Primer Concentrations					
Locus	Forward, µM	Reverse, µM	Labeled, µM	MgCl, mM	DMSO, mM	Annealing °C
Sal1	0.350	0.400	0.040	1.875	-	45 °C
Sal2	0.350	0.400	0.040	1.875	-	48 °C
Sal3	0.350	0.400	0.040	1.875	-	48 °C
Sal4	0.350	0.400	0.040	1.875	-	52 °C
Sal6	0.350	0.400	0.040	1.875	-	55 °C
SR7-2	0.300	0.350	0.035	1.875	-	56 °C
SR7-25	*0.300*	*0.350*	*0.035*	*1.875*	-	*56 °C*
Sr7-7	0.300	0.350	0.035	1.875	-	56 °C
Sma7	0.350	0.400	0.040	1.500	0.0025	54 °C
Seb33	*0.150*	*0.200*	*0.020*	*1.500*	*0.0025*	*54 °C*
Sma5	0.200	0.250	0.025	1.875	-	54 °C
Spi4	0.200	0.250	0.025	1.875	-	54 °C
Spi6	0.200	0.250	0.025	1.875	-	58 °C
Spi10	0.200	0.250	0.025	1.875	-	60 °C
Spi12	0.200	0.250	0.025	1.875	-	58 °C
Sth3B	0.200	0.250	0.025	1.875	-	60 °C
Sma11	*0.350*	*0.400*	*0.040*	*1.875*	-	*52 °C*

Table A-2. Assignment proportions for all geographic groups from the program CBayes v.2.5.

Individuals are assigned from the population at the top to those on the left. Self assignment rates are bold face and standard deviations for each assignment rate are reported to the right.

	QCI	CSS	YAK	COR	KOD	SHU	AKU	ALE	WAL	SBS	CBS
QCI	**0.89** (0.06)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.01 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.00)	0.00 (0.01)
CSS	0.01 (0.02)	**0.98** (0.02)	0.00 (0.01)	0.01 (0.02)	0.00 (0.01)	0.00 (0.01)	0.01 (0.02)	0.00 (0.00)	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)
YAK	0.08 (0.06)	0.01 (0.01)	**0.98** (0.02)	0.01 (0.02)	0.00 (0.01)	0.00 (0.01)	0.05 (0.04)	0.00 (0.00)	0.00 (0.01)	0.00 (0.00)	0.00 (0.01)
COR	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	**0.89** (0.05)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.01 (0.01)
KOD	0.00 (0.01)	0.00 (0.00)	0.00 (0.01)	0.05 (0.03)	**0.97** (0.03)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.01 (0.02)	0.00 (0.01)	0.01 (0.01)
SHU	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.01)	0.01 (0.02)	**0.97** (0.02)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.01 (0.01)
AKU	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	**0.91** (0.05)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.02 (0.02)
ALE	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	**0.97** (0.03)	0.00 (0.01)	0.11 (0.02)	0.00 (0.01)
WAL	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.01 (0.02)	0.01 (0.02)	**0.96** (0.04)	0.01 (0.01)	0.00 (0.01)
SBS	0.00 (0.01)	0.00 (0.01)	0.00 (0.00)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.02)	0.01 (0.02)	0.01 (0.02)	**0.95** (0.04)	0.01 (0.02)
CBS	0.00 (0.01)	0.00 (0.00)	0.00 (0.01)	0.02 (0.03)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.01 (0.01)	0.00 (0.01)	0.02 (0.03)	**0.95** (0.04)

Table A-3. Assignment proportions with the leave-one-out procedure (individual jackknife) in GENECLASS2. Individuals being assigned are removed from their population's allele calculations.

	QCI	CSS	YAK	COR	KOD	SHU	AKU	ALE	WAL	SBS	CBS
QCI	**0.2126**	0.1021	0.1003	0.0307	0.0398	0.0281	0.0021	0.0365	0.0137	0.0123	0.0348
CSS	0.1728	**0.4775**	0.2811	0.0919	0.0177	0.0220	0.0441	0.0077	0.0249	0.0100	0.0144
YAK	0.2153	0.2153	**0.4661**	0.0610	0.0219	0.0141	0.0523	0.0119	0.0013	0.0137	0.0144
COR	0.0352	0.0759	0.0443	**0.3193**	0.0934	0.0996	0.0420	0.0659	0.0316	0.0797	0.0757
KOD	0.0249	0.0131	0.0135	0.0643	**0.0940**	0.0562	0.0429	0.0507	0.1362	0.0998	0.0317
SHU	0.0698	0.0225	0.0203	0.0863	0.1099	**0.3323**	0.0775	0.0852	0.0429	0.0939	0.1055
AKU	0.0594	0.0109	0.0108	0.0485	0.0926	0.0492	**0.2076**	0.0813	0.0867	0.0641	0.0505
ALE	0.0563	0.0186	0.0180	0.0705	0.1304	0.0865	0.1413	**0.2448**	0.0795	0.1379	0.1824
WAL	0.0099	0.0104	0.0028	0.0179	0.0564	0.0382	0.0734	0.0797	**0.2044**	0.0876	0.0431
SBS	0.1019	0.0343	0.0114	0.1027	0.2294	0.1459	0.2202	0.1976	0.2879	**0.2700**	0.1366
CBS	0.0299	0.0100	0.0266	0.0917	0.0932	0.1127	0.0789	0.1283	0.0709	0.1149	**0.2982**

Table A-4. Assignment proportions with low frequency alleles pooled.

Assignment proportions with the leave-one-out procedure using GENECLASS2 with low frequency (<0.02) alleles pooled with the most abundant alleles at their locus.

	QCI	CSS	YAK	COR	KOD	SHU	AKU	ALE	WAL	SBS	CBS
QCI	**0.3322**	0.0786	0.0616	0.0387	0.0316	0.0286	0.0116	0.0143	0.0114	0.0175	0.0233
CSS	0.1295	**0.5613**	0.2380	0.0552	0.0277	0.0105	0.0524	0.0011	0.0131	0.0185	0.0107
YAK	0.1117	0.1836	**0.5841**	0.0496	0.0061	0.0216	0.0250	0.0048	0.0022	0.0099	0.0077
COR	0.0731	0.0433	0.0207	**0.2535**	0.0678	0.1372	0.0827	0.0767	0.0382	0.0757	0.0877
KOD	0.0201	0.0238	0.0132	0.0536	**0.1631**	0.0625	0.0630	0.0805	0.1107	0.0870	0.0424
SHU	0.0718	0.0157	0.0142	0.1553	0.1524	**0.2473**	0.0842	0.1025	0.0465	0.0923	0.1115
AKU	0.0333	0.0222	0.0153	0.0713	0.0667	0.0810	**0.2407**	0.0706	0.0616	0.0655	0.0569
ALE	0.0346	0.0069	0.0107	0.0812	0.1396	0.0934	0.1059	**0.2685**	0.0850	0.1258	0.1080
WAL	0.0571	0.0124	0.0067	0.0219	0.0530	0.0547	0.0816	0.0924	**0.2596**	0.1099	0.0409
SBS	0.1031	0.0273	0.0127	0.0773	0.1595	0.1253	0.1396	0.1525	0.2428	**0.2670**	0.1280
CBS	0.0169	0.0098	0.0162	0.1082	0.0923	0.1068	0.0757	0.1113	0.0951	0.0997	**0.3603**