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Co-existing populations of Pacific ocean perch, *Sebastes alutus*, in Queen Charlotte Sound, British Columbia

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Abstract Variation at five microsatellite loci (*Sal*1, *Sal*2, *Sal*3, *Sal*4 and *Sal*5) was examined in approximately 1300 Pacific ocean perch (*Sebastes alutus*) sampled from 14 coastal sites in British Columbia, Canada. Mean observed heterozygosities by locus ranged from 71% to 88%, and by sample ranged from 75% to 84%. Theta values ranged from 0 to 0.04 over the five loci, and averaged 0.015. Among Pacific ocean perch samples, θ ranged from 0.001 to 0.056. Canonical discriminate analysis of multilocus genotypes and neighbour-joining analysis of pairwise genetic distances between samples both indicated the presence of three populations, one off the west coast of Vancouver Island (the Vancouver Island population) and two co-existing populations in Queen Charlotte Sound, Dixon Entrance and along the west coast of the Queen Charlotte Islands (the eastern and western QCI populations). Pacific ocean perch of the eastern and western QCI populations were caught in close proximity to each other, but individual samples showed little evidence of admixture. Fall and spring samples collected within geographic areas were genetically similar, indicating seasonally stable population structure. Restricted gene flow between the Vancouver Island and the two more northerly populations may result from limited adult dispersal and larval retention within the California Current and Alaska Gyre, respectively, but the presence of two populations within Queen Charlotte Sound cannot be explained entirely by larval retention hypotheses. The presence of two Pacific ocean perch populations in central British Columbia has implications for fisheries management.

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Introduction

Conservation of marine organisms is a serious challenge for fisheries management (Stepien 1995; Waples 1998). Exploitation acts to reduce population biomass, so that populations may have less ability to respond to the large variations in recruitment and natural mortality experienced in the marine environment. Often little is known of the geographic scale over which dispersal of adults and/or pelagic larvae, assumed to be the important determinants of population dynamics and population genetics, occur. An increasing number of studies on marine fish inhabiting continental shelf regions have indicated that early concepts of extensive gene flow throughout a continuous and homogeneous marine environment were oversimplified. Instead, small-scale bathymetry and associated local currents may be as effective in limiting larval dispersal and gene flow as large-scale oceanic currents are in facilitating exchange (Wilson and Waples 1984; Stepien 1999). The geographic delineation of population structure in marine species is an important component of fisheries management, enabling identification of regions in which harvest may impact more than one population with differing levels of productivity.

Rockfish of the genus *Sebastes* constitute an important component of the marine ecosystem in the northeastern Pacific Ocean, both in terms of the number of extant species and their ubiquitous presence in habitats ranging from shallow coastal waters to the deeper expanses of the continental shelf. In Canada, for example, more than 20 species are harvested in commercial and recreational fisheries. *Sebastes* are live-bearing, slow-growing, long-lived fish, with the bulk of their reproductive capacity concentrated in the ranks of large females aged 25 years and older (Leaman 1991). Because strong year classes tend to occur infrequently, recovery of abundance from overfishing can be a slow and uncertain process (Leaman 1991; Gunderson 1997).

Pacific ocean perch (*Sebastes alutus*), a dominant species of the deep-water "slope" rockfish assemblage,

has been extensively fished over its North American range from California to Alaska since 1940 (Quast 1972; Westrheim et al. 1972; DFO 1999). Pacific ocean perch, along with other slope rockfishes, was heavily targeted during the late 1960s and early 1970s, and was depleted in major fishing areas by the late 1970s (Gunderson 1977; Archibald et al. 1983). Abundances in some, but not all, of these areas have subsequently recovered (Ianelli and Heifetz 1995; Richards et al. 1997). Between 1980 and 1996, the average annual catch in British Columbia was 5680 t (approximately 5.7 million pieces) (DFO 1999). The species is distributed continuously, but at varying abundances, along the outer continental shelf and upper continental slope in waters ranging in depth from 40 to 640 m (Westrheim 1973). Westrheim (1975) has described a "shallow-water" and "deep-water" form of Pacific ocean perch off the south-west coast of Vancouver Island, which differ in timing of gonadal development and in seasonal availability to fishing gear.

In British Columbia, breeding of Pacific ocean perch occurs during fall (September–October) (Westrheim 1975) and parturition occurs during spring (March off Vancouver Island and somewhat later in Queen Charlotte Sound) (Westrheim 1975; DFO 1999). Little is known of the degree of dispersal that takes place during the 3- to 6-month period during which the larvae are planktonic, or during subsequent movements of juveniles which may remain pelagic until the second or third year of life. Larval movement may be strongly affected by nearshore coastal flow dynamics, whereas pelagic juveniles may be influenced by both nearshore and offshore flows (Moser and Boehlert 1991).

Years of highly successful Pacific ocean perch recruitment tend to be synchronous on a coastwide basis (Hollowed et al. 1987; Ralston and Howard 1995), but the degree to which this results from uniform oceanographic conditions or from broad-scale recruitment from localised successful spawning is not known. However, aggregations of Pacific ocean perch depleted by exploitation do not rebuild rapidly once exploitation is reduced, even when they are in the vicinity of other lightly exploited aggregations. The localised nature of population dynamics and demographics indicates that larval dispersal is limited, possibly to a degree that would enable genetic differentiation on a smaller geographic scale than previously detected. Alternately, even episodic periods of limited larval dispersal would tend to maintain large-scale genetic homogeneity.

Rational management of Pacific ocean perch to maintain abundance and conserve existing biodiversity requires an understanding of the genetic structure of the species. A survey of genetic variation at allozyme loci revealed low levels of population differentiation among samples collected from coastal Washington to the Bering Sea (Seeb and Gunderson 1988). In that study, hampered by a low level of polymorphism at the allozyme loci, there was evidence for genetic differentiation between the Pacific ocean perch populations of Washington and Alaska, but only weak support for a proposed cline in allele frequencies over the intervening waters, in spite of extensive sampling in coastal British Columbia. Multiple samples collected from within several regions of major Pacific ocean perch habitat, including Dixon Entrance and Moresby, Mitchell's and Goose Island gullies in Queen Charlotte Sound, displayed greater genetic heterogeneity than would be expected if genetic differentiation throughout British Columbia was clinal. These results supported the analysis of Gunderson (1972), who found evidence of biologically distinct groups of Pacific ocean perch within Queen Charlotte Sound in samples from commercial fishery and research cruise catches.

In this study we test the hypothesis that population structure in Pacific ocean perch within British Columbia fits a model of isolation-by-distance, particularly in the Queen Charlotte Sound and Dixon Entrance regions. The analysis is based on a survey of five microsatellite loci in 14 samples of adult Pacific ocean perch collected from waters off Vancouver Island, in Queen Charlotte Sound along the west coast of the Queen Charlotte Islands and in Dixon Entrance. A positive result would indicate that gene flow among adult Pacific ocean perch aggregations occurs and is limited primarily by distance. A negative result and the finding of relatively large genetic differentiation over small geographic distances would be consistent with the suggestion that adult aggregations can reflect genetically as well as demographically distinct units, among which gene flow is restricted by factors other than distance.

Materials and methods

Sample collection and PCR

Over 1200 Pacific adult ocean perch, *Sebastes alutus*, were sampled from 14 sites between April 1997 and March 1998 (Table 1). Samples were collected primarily in fall (September–November) or spring (March–June). Sample sites ranged from the north-western tip of the Queen Charlotte Islands to the south-western tip of Vancouver Island (Fig. 1). At each location, dorsal fins from between 43 and 176 fish from a single bottom net haul were frozen by observers or research staff aboard commercial fishing vessels. The sample from Estevan was an exception, it was taken from a midwater trawl (153 m depth). DNA was extracted using a chelex procedure (Small et al. 1998) and used in the PCR (polymerase chain reaction) amplification of alleles at five microsatellite loci, *Sal*1–5, isolated from Pacific ocean perch and amplified as outlined by Miller et al. (2000).

Gel electrophoresis and fragment analysis

PCR products were size fractionated on 16×17 cm non-denaturing polyacrylamide gels, and visualised by staining with ethidium bromide (0.5 mg ml^{-1}) in water and illuminating with ultraviolet light. All gels were run for 15–17 h at 80–85 V. Analysis of *Sal*1, *Sal*3, *Sal*4 and *Sal*5 was conducted on 10% acrylamide gels, and of *Sal*2 was conducted on 12% acrylamide gels. Twenty-nine lanes per gel were loaded, four with DNA size ladders and 25 with DNA samples from the fish to be analysed (including a standard fish run on every gel to determine precision of estimation). Then, 1-kb (kilobase) ladder DNA (GibcoBRL) was placed in an outside lane,

Table 1 Locations and dates of Pacific ocean perch (*Sebastes alutus*) sample collections (*n* number of fish sampled from each location)

Sample no.	Sample name	Date	*n*	Latitude	Longitude
1	Langara – inside	9 Sep 1997	92	54 17.4	133 27.6
2	Langara – outside	10 Sep 1997	94	54 11.4	133 45.9
3	Rennell Sound	20 Sep 1997	95	53 18.5	133 04.4
4	Flamingo	21 Sep 1997	91	52 08.5	131 31.0
5	Moresby Gully – N	8 May 1997	94	52 09.0	130 47.8
6	Moresby Gully – N	21 Apr 1997	95	52 00.9	130 46.0
7	Moresby Gully – S	16 Jun 1997	94	51 50.9	130 38.7
8	Moresby Gully – S	3 May 1997	177	51 43.9	130 47.1
9	Moresby Gully – S	21 Sep 1997	91	51 43.6	130 47.6
10	Goose Island Gully	27 Apr 1997	95	51 19.4	129 03.7
11	Goose Island Gully	3 Mar 1998	73	51 12.3	128 52.9
12	Goose Island Gully	14 Nov 1997	92	51 11.7	128 29.6
13	Estevan	10 Sep 1997	95	49 35.1	127 17.7
14	SW Vancouver Isl.	25 Apr 1997	49	48 46.5	126 25.9

and 20-bp (basepair) ladder DNA (Gensura Labs, Del Mar, Calif.) was placed in three lanes spaced evenly across the gel.

Gels were scanned at a 1024×1024 pixel density with a Kodak charge-coupled device camera with low light capability and a yellow filter. Images were analysed using BioImage Whole Band software (Millipore Imaging Systems, Ann Arbor, Mich.), with the size of the amplified microsatellite alleles reported to the nearest basepair according to the molecular size grid created with the 20-bp size ladders.

We identified alleles using a binning procedure (Gill et al. 1990) because of the uncertainty in estimation of allele size based on the 20-bp size grid. *Sal*1, *Sal*2, *Sal*4 and *Sal*5 were tetranucleotide repeats, and *Sal*3 was a pentanucleotide repeat (Miller et al. 2000). Peaks in the allele frequency distribution for each locus were used to identify the main alleles. Bin widths generally corresponded to the microsatellite repeat units, and were defined so that the modal size estimate for an allele was centred in each bin. Alleles were identified by the lower limit of the bin in which they were encompassed. Only for *Sal*1 did the frequency distribution of alleles

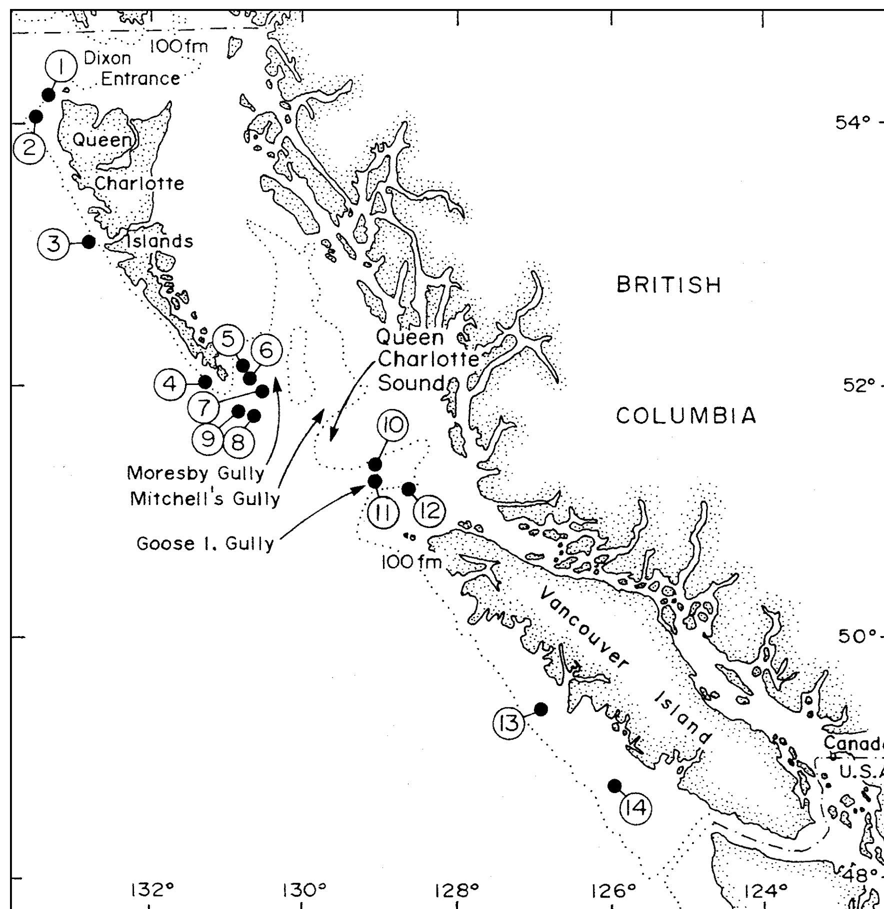

Fig. 1 Sampling locations of Pacific ocean perch (*Sebastes alutus*) analysed at five microsatellite loci. The sample name and latitude and longitude associated with the numbers on this map are given in Table 1

indicate any irregularity in allele sizes, necessitating a single 6-bp bin encompassing fragments between 175 and 180 bp in length. Precision of estimation of allele size was evaluated with the standard fish analysed for each locus.

Data analysis

Genotypes at each locus in each of the 14 samples were tested for departure from Hardy–Weinberg equilibrium using GENEPOP version 3.1 (Raymond and Rousset 1995). Tests of genetic differentiation using all pairwise comparisons were conducted using GENEPOP with the dememorisation number set at 1000 and running 50 batches for each test with 1000 iterations per batch. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989). Weir and Cockerham's (1984) θ (F_{st}) values were calculated with the Genetic Data Analysis (GDA) program of Lewis and Zaykin (2000) in an analysis of population subdivision. Standard deviations associated with the θ estimates were determined by jack-knifing over samples. F_{st} estimates were used to describe population structure because of the lack of bias and low sampling variance associated with F_{st} calculated for microsatellite data on samples of the size achieved in this study (Ruzzante 1998). A lack of information on the mutation processes at rockfish microsatellite loci precluded the use of alternate parameters that are estimated under the assumption of single-step-mutation (SSM) models.

Locus by locus tests of genic differentiation were conducted using GENEPOP for all pairwise comparisons of the 14 Pacific ocean perch samples. The 14 samples were combined into three population samples based on genetic similarities, and genic differentiation among the three populations was also assessed with GENEPOP. The SAS (1989) CANDISC program was used to perform canonical discriminate analysis on the multilocus genotypes of perch from all 14 samples. GDA was used to construct neighbour-joining dendrograms of sample relationships based on pairwise values of both Nei's (1972) genetic distance and θ. Hierarchical gene diversity analyses were conducted with GDA to assess genetic variation among three genetic population groups identified in the neighbour-joining dendrogram and by discriminate analysis, between sampling seasons (spring and fall) within population, and among samples within population and season.

Two types of tests were conducted to examine the geographic structure of observed genetic variation. For each of the five microsatellite loci, the correlation between the frequency of the common allele in each sample and latitude was examined to detect clinal variation in allele frequencies. In addition, evidence for isolation-by-distance among populations was examined by a regression of linearised genetic distance [$\theta/(1–\theta)$; Slatkin 1995] on geographic distance for pairwise comparisons between samples (Mantel 1967). Geographic distances between pairs of sample locations were calculated using the SEAPLOT program (Advanced Marine Technology, Seattle, Wash.).

Results

Precision of estimation of allele size

*Sal*1 possessed 25 alleles, ranging from 107 and 209 bp in size, *Sal*2 possessed 17 alleles, ranging from 81 to 145 bp, *Sal*3 possessed 18 alleles, ranging from 88 to 173 bp, *Sal*4 possessed 18 alleles, ranging from 81 to 149 bp and *Sal*5 possessed 16 alleles, ranging from 81 to 141 bp. Standard deviations of the estimates of allele size for the heterozygous standard fish analysed at each locus ranged from 0.39 to 1.06 bp (Table 2). For all alleles carried by the standard fish, 100% of the estimated sizes fell within either 3- or 4-bp intervals. Thus, the 4-bp bins used for each locus (except *Sal*3, which had 5-bp bins due to the pentanucleotide repeat) were well supported.

Table 2 *Sebastes alutus*. Precision of estimation of allele size (bp) for standard fish analysed repeatedly at each locus, with the fish run only once on each gel (*n* number of gels where allele sizes for the fish were estimated). Mean allele size is shown with the standard deviation in *parentheses*

Locus	*n*	Allele size	Range
*Sal*1	37	176.9 (1.06)	175–178
	37	124.6 (0.54)	124–126
*Sal*2	58	121.9 (0.72)	121–124
	58	101.6 (0.59)	100–103
*Sal*3	49	139.4 (0.58)	138–141
	49	110.3 (0.50)	109–111
*Sal*4	56	125.8 (0.76)	124–127
	56	94.5 (0.60)	93–96
*Sal*5	44	121.8 (0.61)	121–124
	44	106.1 (0.39)	105–107

Genetic variation within samples

All five microsatellite loci were polymorphic in all 14 Pacific ocean perch (*Sebastes alutus*) samples. Average heterozygosity by locus was 87.9% at *Sal*1, 77.5% at *Sal*2, 83.3% at *Sal*3, 79.4% at *Sal*4 and 70.6% at *Sal*5. Observed heterozygosity over all loci ranged from 75.2% to 83.8% among the 14 samples, with no apparent geographic pattern in observed variation. Genotype frequencies in each sample location were in Hardy–Weinberg equilibrium, with the exception of *Sal*4 in the south-west Vancouver Island sample and *Sal*5 in the Langara outside sample, both of which exhibited an excess of homozygotes (both $P=0.004$). There was no significant genotypic disequilibrium between loci within samples, except for the south Moresby sample collected in September, in which both *Sal*1 and *Sal*3, and *Sal*1 and *Sal*5, displayed significant departures from linkage equilibrium (both $P=0.000$).

Genetic structure among samples

For locus-by-locus tests of genic differentiation, allele frequencies differed significantly at zero to four of the five loci in pairwise comparisons between samples (Table 3). Allele frequencies at *Sal*2 were homogeneous among samples, and did not differ significantly in any pairwise comparison. The estimated values of θ by locus ranged from 0.000 at *Sal*2 to 0.041 at *Sal*5, and the values for *Sal*1, *Sal*3 and *Sal*5 were significantly greater than zero (Table 4). The mean pairwise θ value between Pacific ocean perch samples was 0.015 (0.008 SD), with individual values ranging from 0.001 to 0.056.

Within Moresby Gully, there was no significant difference in allele frequencies at any locus between the two

Table 3 *Sebastes alutus*. Number of loci out of five at which allele frequencies differed significantly in pairwise comparisons of Pacific ocean perch samples, with significance values evaluated using sequential Bonferroni adjustment. Values *above the diagonal* are for comparisons of samples from different populations and *below the diagonal* are for comparisons of samples from the same population

Population, sample	Sample													
	13	14	1	3	4	7	8	9	2	5	6	10	11	12
Vancouver Island														
13 Estevan	–	2	2	2	2	2	3	2	3	3	2	3	3	3
14 SW Vancouver Isl.	1	–	3	3	2	3	3	3	2	3	3	2	4	1
Western QCI														
1 Langara – inside			–						3	1	1	2	2	2
3 Rennell Sound			1	–					2	1	1	2	2	1
4 Flamingo			1	0	–				3	2	1	2	4	2
7 Moresby Gully – S			0	1	0	–			3	3	2	3	3	3
8 Moresby Gully – S			0	1	0	0	–		3	3	3	3	3	3
9 Moresby Gully – S			1	1	1	1	1	–	4	3	4	4	4	4
Eastern QCI														
2 Langara – outside									–					
5 Moresby Gully – N									1	–				
6 Moresby Gully – N									0	0	–			
10 Goose Island Gully									2	1	1	–		
11 Goose Island Gully									2	1	1	0	–	
12 Goose Island Gully									2	2	1	1	0	–

samples collected during spring north of 52° latitude (samples 5 and 6), nor between the two samples collected during spring south of 52° (7 and 8) (Fig. 1; Table 3). However, allele frequencies were generally significantly different at three of the five microsatellite loci (*Sal*1, *Sal*3, *Sal*5) in pairwise comparisons between the north and south Moresby Gully samples (Table 3). After the undifferentiated pairs of north and south Moresby spring samples were pooled for use in examination of the geographic basis of population structure (described below), they remained significantly different in allele frequencies at all three loci.

The two spring samples from Goose Island Gully (10 and 11) were collected a year apart, but allele frequencies did not differ significantly at any locus and the samples were combined for geographic analysis. All remaining pairs of samples that were not differentiated by allele frequencies at any locus were collected in different sample locations or different seasons and were kept separate. These included the Rennell Sound (3) and Flamingo (4) samples collected off the west coast of the Queen Charlotte Islands and the Langara inside fall (1) and the two south Moresby spring samples. In contrast, the two fall samples from either side of Langara Point in Dixon Entrance (1 and 2), collected on successive days from the same boat at locations 35 km apart, differed significantly at the same three loci (*Sal*1, *Sal*3 and *Sal*5) as the north and south Moresby samples (Table 3). Thus, the Langara samples were not combined.

Table 4 *Sebastes alutus*. Theta values of five microsatellite loci for Pacific ocean perch samples collected in British Columbia. The standard deviation for each θ estimate is given in *parentheses*, and the probability (P) of each estimate is shown. The correlation coefficient (r) and its significance for the relationship of the common allele at each locus with latitude are also given

Locus	θ	P	r	P
*Sal*1	0.021 (0.006)	<0.05	0.17	0.56
*Sal*2	0.000 (0.001)	>0.05	0.14	0.66
*Sal*3	0.007 (0.003)	<0.05	0.47	0.14
*Sal*4	0.004 (0.003)	>0.05	0.11	0.73
*Sal*5	0.041 (0.020)	<0.05	0.44	0.17

The two samples from the west coast of Vancouver Island [SW Vancouver Island (14) and Estevan Point (13)] were distinctive in both the neighbour-joining analysis of Nei's genetic distance and the discriminant analysis of multilocus genotypes, indicating the existence of a Vancouver Island population of Pacific ocean perch (Figs. 2, 3, 4). They differed significantly from all other samples, except one at both *Sal*1 and *Sal*5, and tended to be differentiated at *Sal*3 and *Sal*4 as well (Table 3; Fig. 2).

The remaining samples fell into two groups apparent in both the genetic distance dendrogram (Fig. 3) and the discriminant analysis (Fig. 4). One contained the samples from Goose Island Gully (10, 11, 12), north Moresby Gully (5, 6) and Dixon Entrance [Langara outside (2)] and the other contained samples from south Moresby Gully (7, 8, 9), the west coast of the Queen Charlotte Islands [Rennell Sound (3) and Flamingo (4)] and Dixon Entrance [Langara inside (1)]. The neighbour-joining dendrogram of F_{st} values (not shown) was identical in topology and relative branch length to one based on genetic distance (Fig. 3), except that the positions of the Rennell (3) and Langara inside (1) samples were reversed, and the Goose Island fall sample (12) clustered with Goose Island spring sample 11 before both clustered with Goose Island spring sample 10.

The samples in these two groups, hereafter termed the eastern and western Queen Charlotte Island (QCI) populations, respectively, generally had significantly different allele frequencies at *Sal*1, *Sal*3 and *Sal*5 (Fig. 2). Genetic differentiation within each of the two

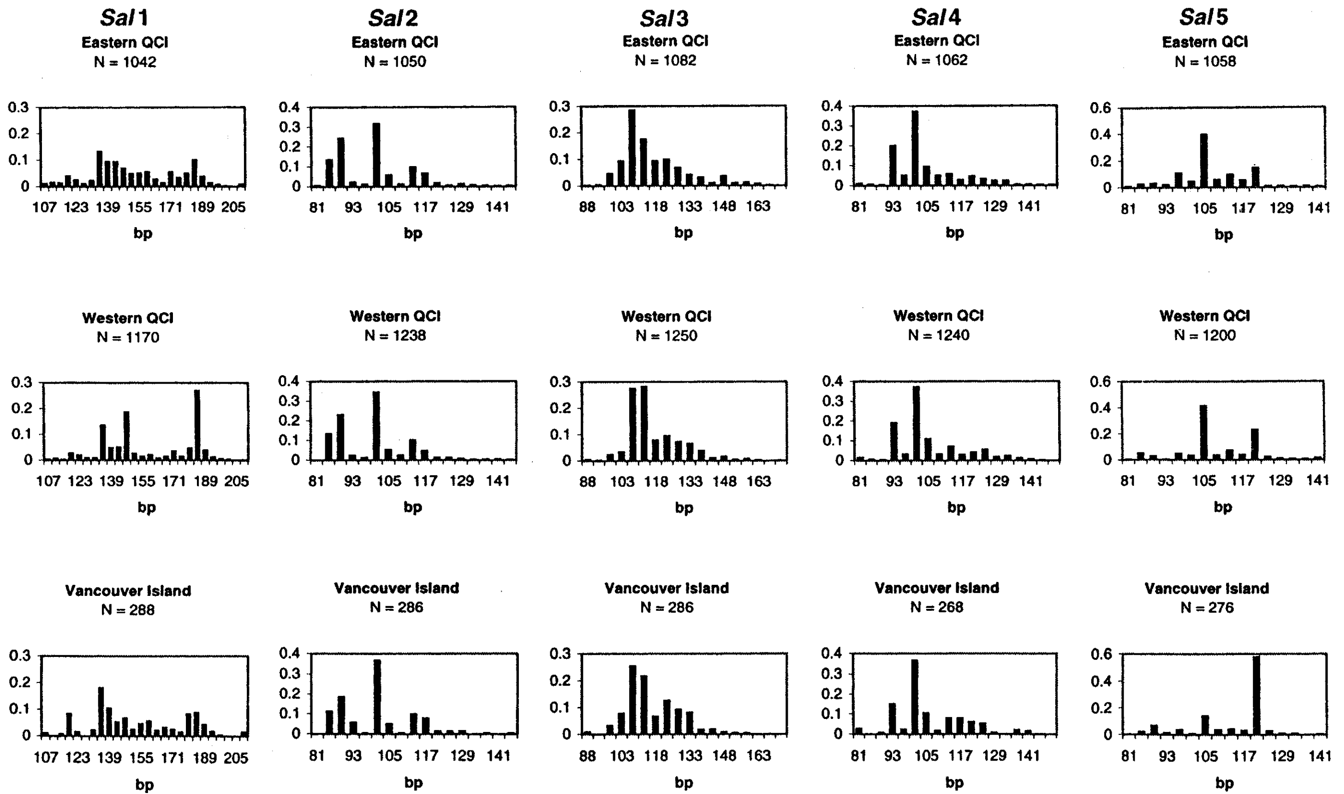

Fig. 2 *Sebastes alutus*. Allele frequency distributions at five microsatellite loci for the Vancouver Island, western Queen Charlotte Island (*QCI*) and eastern QCI populations of Pacific ocean perch in British Columbia. The samples belonging to each of the three populations are identified in Fig. 3. Alleles are identified by length (bp). The number of alleles scored (*n*) at each locus in each population is shown

QCI populations was limited, although intrapopulation samples could differ significantly at up to two loci (Table 3). The average pairwise θ value between samples within populations was 0.006, whereas the average pairwise value across the eastern and western QCI populations was 0.026. Pairwise θ values for comparisons between samples of the Vancouver Island and western QCI populations averaged 0.035, and for comparisons between Vancouver Island and eastern QCI samples averaged 0.037. With all of the fish sampled within each of the three populations pooled, the F_{st} estimate among the three populations was 0.017. Allele frequencies at *Sal*1, *Sal*3 and *Sal*5 differed in all pairwise comparisons among the three populations (all $P < 0.02$), and at *Sal*4 between the Vancouver Island population and each of the Queen Charlotte Island populations (both $P < 0.01$).

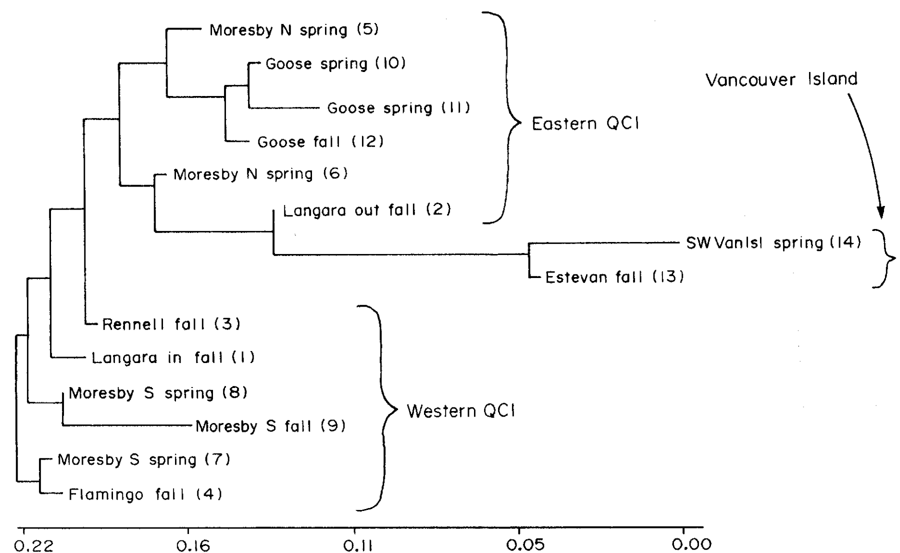

Fig. 3 *Sebastes alutus*. Neighbour-joining dendrogram of 14 Pacific ocean perch samples based on Nei's (1972) distances derived from allele frequencies at five microsatellite loci. Sample numbers are given in Table 1

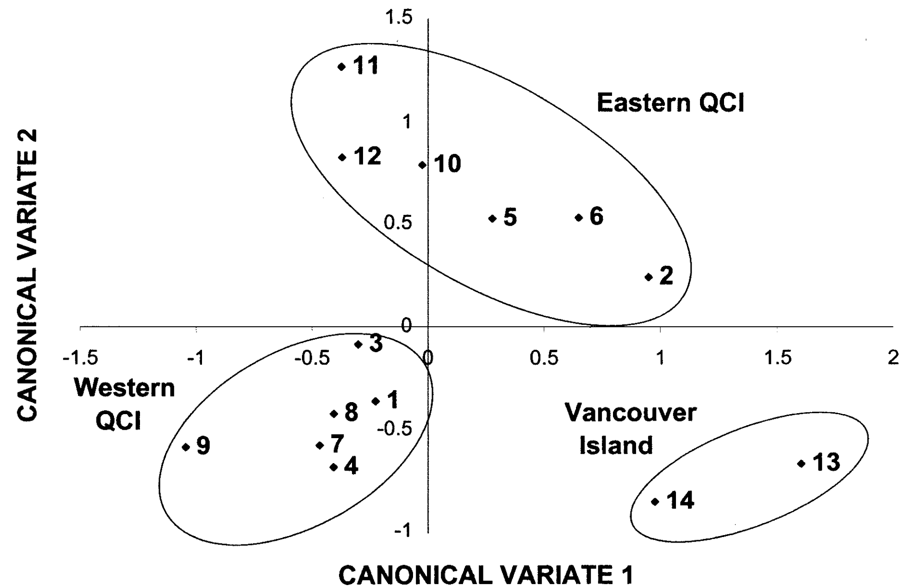

Fig. 4 *Sebastes alutus*. Location of Pacific 14 ocean perch samples on the first two canonical variates resulting from discriminant analysis of individual perch genotypes at five microsatellite loci. *Numbers 1–14* refer to sample collections given in Table 1

Spring and fall samples collected from the same location (Goose Island Gully, south Moresby Gully and west coast Vancouver Island) tended to cluster together, indicating that the same population was sampled in the two seasons at each location. However, the fall sample from south Moresby Gully (9) had unusual allele frequencies at *Sal*4 that distinguished it not only from the spring South Moresby fish but from all other Pacific ocean perch samples of this study (Figs. 3, 4).

In the analysis of gene diversity, season was nested within populations identified from the dendrogram and the discriminant analysis (Vancouver Island, eastern QCI, western QCI), and samples were nested within season. Almost 98% of the variation at the microsatellite loci was contained within samples. The remaining variation was attributed to differences among the three populations ($P<0.01$) and among samples within a population ($P<0.05$), but none was due to season of sample collection ($P>0.1$) (Table 5). Thus, there was no consistent difference between spring and fall samples within either the eastern or western QCI populations, in spite of the distinctiveness of the fall south Moresby sample. The lack of a seasonal effect was also evident in the failure of the samples collected in fall or spring to cluster together within the neighbour-joining and discriminant analyses (Figs. 3, 4).

Geographic basis of population structure

There was no evidence of clinal variation in microsatellite allele frequencies within Pacific ocean perch in British Columbia. The correlation of common allele frequency on latitude was not significant at any locus (all $r<0.5$, all $P>0.10$) (Table 4). Even for *Sal*5, at which the two southernmost Vancouver Island samples possessed much lower frequencies of the common allele than did the remaining samples (Fig. 2), the heterogeneity in allele frequencies within samples from the Moresby and Goose Island gullies of Queen Charlotte Sound, and the two Dixon Entrance samples taken at virtually the same latitude, was sufficient to preclude any suggestion of clinal variation.

There was a weak correlation between pairwise genetic distance (linearised θ) and geographic distance measures among Pacific ocean perch samples ($r^2=0.10$, $P=0.016$) (Fig. 5). This was primarily due to the distinctiveness of the Vancouver Island population, as all

Table 5 *Sebastes alutus*. Hierarchical gene-diversity analysis of 14 samples of Pacific ocean perch from British Columbia at five microsatellite loci. The samples included in each population (Vancouver Island, eastern QCI, western QCI) are indicated in Figs. 3 and 4. Seasons were spring (March–June) and fall (September–November) (Table 1)

Locus	Absolute diversity		Relative diversity			
	Total	Within samples	Within samples	Among samples, within season	Between season, within populations	Among populations
*Sal*1	0.9092	0.8888	0.978	0.011	0.0	0.012
*Sal*2	0.8026	0.8014	0.998	0.001	0.0	0.001
*Sal*3	0.8436	0.8382	0.993	0.003	0.001	0.002
*Sal*4	0.8024	0.7932	0.989	0.010	0.0	0.001
*Sal*5	0.7907	0.7382	0.934	0.021	0.0	0.046
All	4.1484	4.0607	0.979	0.009	0.0	0.012

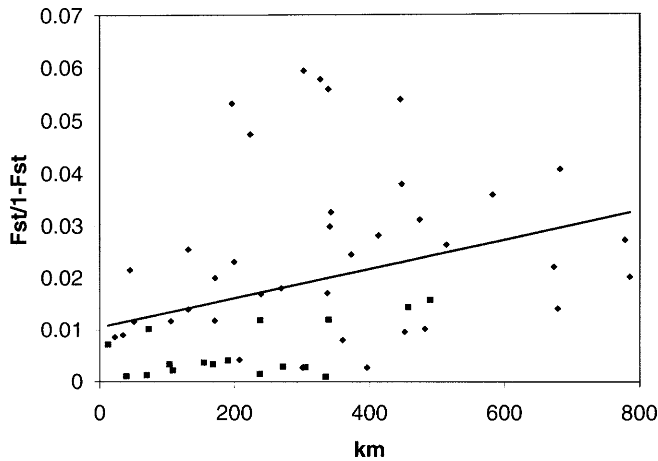

Fig. 5 *Sebastes alutus*. Regression of linearised genetic distance $[F_{ST}/(1-F_{ST})]$ on geographic distance for pairwise comparisons of 11 Pacific ocean perch samples ($r^2=0.10$, $P=0.016$). Values for samples belonging to the same population are indicated by *squares*, those for samples belonging to different populations are indicated by *diamonds*

comparisons between samples >500 km apart involved a comparison between one of the two Vancouver Island samples and a northerly sample from the eastern or western QCI populations. The poor fit of the regression was due to a large number of comparisons, which all involved samples from different populations, in which the genetic differentiation was greater than that expected due to geographic distance alone (Fig. 5). All of the values falling above the fitted regression line represented comparisons of samples from different populations, with the most extreme values resulting from comparisons of the interspersed eastern and western QCI samples.

Discussion

The existence of distinct Pacific ocean perch (*Sebastes alutus*) populations in the waters off Vancouver Island and those surrounding the Queen Charlotte Islands, and even of heterogeneous populations within the waters of Queen Charlotte Sound, had been suggested by several types of biological data, including lack of migration among adult populations, distinct patterns of growth, survival and fecundity, and different parasite profiles (Gunderson 1972, 1977; Westrheim 1975; Leaman and Kabata 1987). However, the degree to which these differences reflected genetic differentiation rather than a phenotypic response to environmental variability was unresolved. Seeb and Gunderson (1988) found that Pacific ocean perch from southern British Columbia were more similar genetically to those of Washington and Oregon than to those of northern British Columbia and Alaska, and also documented genetic heterogeneity within the major gullies of Queen Charlotte Sound. However, the low level of variation observed at allozyme loci precluded the identification of individual Pacific ocean perch populations within British Columbia. The present study of genetic differentiation at highly polymorphic microsatellite loci confirmed the distinction of the Vancouver Island population, and provided strong evidence for the existence of two populations occupying the waters of Queen Charlotte Sound and Dixon Entrance. The hypothesis of a population structure governed by isolation-by-distance for Pacific ocean perch within British Columbia was refuted by both the lack of clinal variation in allele frequencies and the geographic interspersion of genetically distinct samples belonging to the eastern and western QCI populations in Queen Charlotte Sound and Dixon Entrance.

The existence of distinct Pacific ocean perch populations along the continental shelf of the north-eastern Pacific Ocean adds to a growing number of shelf species in which non-linear latitudinal genetic subdivision has been elucidated, but Pacific ocean perch is the first species in which genetically differentiated populations have been found to occupy waters within a similar latitudinal range. Fish from the eastern and western QCI Pacific ocean perch populations apparently remain in separate aggregations throughout their lives, in spite of the wide dispersion of both populations throughout Queen Charlotte Sound and Dixon Entrance during both spring (parturition) and fall (breeding). All samples collected north of Vancouver Island apparently were composed of fish belonging to only one of the two populations, although different samples collected within 35 km of each other on successive days could belong to the two different populations. Genotypic frequencies within samples were in Hardy–Weinberg equilibrium, showing no evidence of the excess of homozygotes that might be expected if samples were mixtures of fish from two populations. Similarly, genotypes in all samples except one were in linkage equilibrium, indicating that the five microsatellite loci were unlinked and providing no evidence of population admixture in individual samples other than the September sample from south Moresby Gully. This study has provided a genetic basis for the biologically distinct groups of Pacific ocean perch within Queen Charlotte Sound first detected by Gunderson (1972).

The level of genetic differentiation detected among the three Pacific ocean perch populations sampled is high for a continental shelf species, especially since two of the populations occupy the same or similar latitudes. The F_{st} value among the three populations, estimated with samples within each population pooled, was 0.017, similar in magnitude to the 0.023 value distinguishing pooled sea bass (*Dicentrarchus labrax*) samples from the western Mediterranean Sea and the north-eastern Atlantic Ocean examined at six microsatellite loci (Naciri et al. 1999). Pairwise F_{st} values estimated between individual samples from the different populations were also similar in the two studies, ranging to 0.056 in the present study and to 0.051 for the Mediterranean and Atlantic sea bass samples. A much lower, but

sometimes significant, level of genetic heterogeneity existed among samples within each of the Pacific ocean perch populations. This may reflect sampling issues (i.e. sample size, sample depth, seasonal migration, sample admixture) or may reflect stable subpopulation structure. Seasonal effects were suggested by the distinctiveness of the fall South Moresby sample, but there was no consistent effect of season on allele frequencies ($P > 0.1$) in either the eastern or western QCI populations, each of which was represented by more than one spring and fall sample. The strongest indication of subpopulation structure was provided by the three Goose Island Gully samples collected over a year, which clustered together in both the neighbour-joining analysis of genetic distance and the discriminant analysis of individual genotypes. Further sampling specifically addressed at each of the factors potentially affecting sample heterogeneity will be required to provide a more detailed understanding of Pacific ocean perch population structure in British Columbia.

Denoting the two Queen Charlotte Sound populations of Pacific ocean perch detected in this study the eastern and western QCI populations suggests a simple geographic basis for population separation that likely does not exist. The south Moresby samples (belonging to the western QCI population) were collected at the same longitudes as the north Moresby samples (belonging to the eastern QCI population) and the two Dixon Entrance samples, one belonging to each of the populations, were caught in extremely close proximity. Both populations may also occupy Mitchell's and Goose Island gullies given the significant genetic heterogeneity detected among replicate samples within all three Queen Charlotte Sound gullies by Seeb and Gunderson (1988). Thus, both populations are likely widely dispersed throughout Queen Charlotte Sound and waters surrounding the Queen Charlotte Islands.

Breeding segregation of the two QCI populations might be mediated by breeding time, geographic location, depth, temperature or some other aspect of spawning activity or habitat. Westrheim (personal communication) has suggested that there is morphological evidence for shallow- and deep-water forms of Pacific ocean perch in Goose Island, Mitchell's and Moresby gullies. It is surprising that the south Moresby sample displaying genotypic disequilibrium at two pairs of loci was sampled during September, at a time when Pacific ocean perch populations might be expected to be partially or completely segregated for breeding. Information on fish size and stage of maturity was not collected for the fish sampled in this study. For most samples, either the starting depth, or both the starting and ending depths, of the haul in which Pacific ocean perch were captured was recorded. However, for the most part, the depth range covered within hauls was greater than the variability among hauls, precluding a precise determination of the depth at which the fish were actually caught or a comparison of allele frequencies by depth.

Since members of the two QCI Pacific ocean perch populations apparently not only are segregated during breeding but exist in separate adult aggregations throughout the year, a mechanism preventing larval and juvenile intermixing likely exists. Surface and depth current conditions within both Queen Charlotte Sound and Dixon Entrance are complex and, because they are influenced by wind, freshwater runoff and tidal factors, likely vary on an annual as well as seasonal basis (Thomson 1981; Scott 1995). The major offshore current is the northward flowing Alaska Current, but small-scale gyres and upwelling currents have been identified in Queen Charlotte Sound and Dixon Entrance. Moreover, the surface flow through Hecate Strait (separating the Queen Charlotte Islands and mainland British Columbia just north of Queen Charlotte Sound) reverses from the prevailing northward direction in winter to a southerly flow during summer, when larval Pacific ocean perch are in the water column. However, while the degree of hydrographic complexity might account for larval retention and the genetic isolation of Pacific ocean perch in the region from populations to the south and north, it does not easily explain the existence of two larval groups within the region. It is possible that segregation of the two larval groups is maintained by the active selection of different current environments (temperature, depth) by the pelagic larvae, which are born live and relatively well developed, or of different benthic environments by the juveniles postsettlement. Active vertical migration in the water column is believed to influence the transport of rockfish larvae from offshore to nearshore waters (Wing et al. 1998), and active migration of late larval Dover sole (*Microstomus pacificus*) to specific inshore nursery areas has been observed (Toole et al. 1997).

Sympatric larval populations of rainbow smelt (*Osmerus mordax*) in the St. Lawrence River estuary belong to two different phylogenetic races of smelt whose genetic differentiation preceded their invasion of a common habitat (Pigeon et al. 1998). Neither allozyme (Seeb and Gunderson 1988) nor the microsatellite data of the present study indicate that the eastern and western QCI perch represent independent phylogenetic lineages. However, mitochondrial DNA (mtDNA) analysis of the rosethorn rockfish (*Sebastes helvomaculatus*) indicated the presence of independent phylogenetic lineages in the Gulf of Alaska and in southern waters off Vancouver Island, Washington and Oregon (Rocha-Olivares and Vetter 1999).

In three other continental shelf species of the northeastern Pacific Ocean, *Sebastolobus alascanus*, *S. altivelis* and *Microstomus pacificus*, a lack of evidence for isolation-by-distance (i.e. non-linear genetic differentiation along a latitudinal gradient from Alaska to California) was detected in the apparent absence of phylogenetic differentiation, and attributed to larval retention within coastal currents (Stepien 1999). Retention of larvae within each of two vastly different hydrographic regimes dominated by the California

Current off southern Vancouver Island and the Alaska Gyre along the remainder of the British Columbia coastline may account for the differentiation between the Vancouver Island Pacific ocean perch population and those to the north, but not for the existence of more than one Pacific ocean perch population within central British Columbia.

The south-west Vancouver Island sample of this study came from a bottom trawl at depths (155–207 fathoms or 279–373 m) at which the "shallow-water" and "deep-water" populations might both be present (Westrheim 1973). The Estevan Point sample collected in September, unusual in that it was taken in a midwater trawl at a depth of 85 fathoms (153 m), was presumably from the shallow-water population. The two Vancouver Island samples were genetically similar, although had significantly different allele frequencies at *Sal*1 ($P=0.000$). The only evidence for the possible presence of fish from two populations in the south-west Vancouver Island sample was an excess of homozygotes at one locus, *Sal*4. Further sampling over a range of depths will be required to determine if two or more Pacific ocean perch populations are present.

Rockfish of the north-eastern Pacific Ocean apparently are characterised by low levels of variability at allozyme loci (Johnson 1973; Wishard et al. 1980; Seeb and Gunderson 1988) but extremely high haplotypic variability in mtDNA, albeit with low levels of nucleotide diversity (Rocha-Olivares and Vetter 1999). The existence of only one or a few genetically divergent haplotype lineages, but high numbers of very similar haplotypes within each lineage, has been observed in numerous marine species of the region and suggests that abundances of these species have increased during the Pleistocene era (Grant and Bowen 1998; Stepien 1999). The high level of polymorphism at microsatellite loci observed in the present study is consistent with the suggestion that the abundance of Pacific ocean perch increased during the Pleistocene, new microsatellite alleles arising through mutation having been retained in this species in the same manner as new mtDNA haplotypes. While the historical record provided by distinct mtDNA lineages within and between species has been invaluable in the reconstruction of *Sebastes* phylogeny and phylogeography (Rocha-Olivares et al. 1999a,b), the intraspecific multitude of similar haplotypes has hindered examination of current levels of genetic exchange, especially given the sample sizes associated with typical mtDNA analyses (Rocha-Olivares and Vetter 1999; Stepien 1999). Use of newly developed microsatellite DNA techniques (Wimberger et al. 1999; Miller et al. 2000) will facilitate evaluation of intraspecific genetic structure within the vast assemblage of rockfish species.

Grant and Bowen (1998) outlined lessons to be learned from the shallow evolutionary lines typical of marine organisms, apparent in the North Pacific *Sebastes* that have been studied to date. In spite of the stability implied by their large population sizes, evidence from both the fossil record (Fitch 1969) and mtDNA analyses indicates that many marine species fluctuate greatly in abundance, even to the point of local or regional extinction, in response to small- and large-scale climate changes. Thus, they are fragile entities even in the absence of exploitation, due at least in part to variation in recruitment success among individuals within and between years, which limits not only population size but also the retention of genetic variability. Excessive harvest not only increases the likelihood of losing genetic variation through reduction of population size, but, by reducing the mean age of individuals, increases the risk that short-term (3–5 year) recruitment failure could lead to local extinction.

The present study supports the current practice of independent assessment and fishery management for Pacific ocean perch off the coast of Vancouver Island and those to the north in Queen Charlotte Sound and waters surrounding the Queen Charlotte Islands. Demarcation of the boundary between the Vancouver Island and QCI populations will require additional sampling between the Estevan Point and Goose Island Gully sites sampled in this study, particularly at locations such as Triangle Island where Pacific ocean perch are harvested. The identification of two independent populations of Pacific ocean perch within Queen Charlotte Sound and Dixon Entrance confirms earlier indications of genetic heterogeneity, but complicates the tasks of stock assessment and fishery management for the region. Within Queen Charlotte Sound the two populations are, to at least some degree, distinct given their different growth characteristics (Gunderson 1972). Elucidation of the spatial or temporal means by which the two populations remain segregated during breeding, and presumably during larval transport and recruitment to a primarily demersal existence, are necessary, although such studies are hindered by the difficulty in identifying *Sebastes* larvae to species (but see Moser 1996; Rocha-Olivares 1998). Given the close proximity of adult aggregations of the two types at various locales throughout the Sound, separate assessment and management will be impractical until the biology of Pacific ocean perch is better understood. If the two populations within Queen Charlotte Sound differ in their depth distributions and availability for harvest in a manner similar to those off the south-west coast of Vancouver Island (Westrheim 1973), independent management may become possible.

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