AN ABSTRACT OF THE DISSERTATION OF

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Title: "Spatial and Temporal Scales of Genetic Change in Two Overfished Rockfishes"

Abstract approved:

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Michael A. Banks

Pacific rockfishes (genus *Sebastes*) have attracted wide scientific and public interest from an evolutionary, fishery and conservation standpoints. This dissertation addresses several hypotheses involving spatial and temporal scales of genetic change in two overfished rockfishes, darkblotched (*S. crameri*) and canary rockfish (*S. pinniger*), using statistical analyses of genetic variation within microsatellite DNA. First, I isolate and characterize 9 polymorphic microsatellites from canary rockfish useful in at least 13 congeneric species. Second, I investigate spatial differentiation in darkblotched rockfish, and how pooling samples with few individuals affects estimates of genetic parameters. Results indicate (*i*) significant correlation between pairwise genetic and geographic distances (isolation-by-distance) among samples, implying that scales of larval dispersal are short, and (*ii*) using an absolute genetic distance to pool samples increases overall genetic differentiation and resolves deviations from Hardy-Weinberg equilibrium without creating data artifacts. Third, I report the variance effective population size (N_e) for darkblotched rockfish by

measuring allele frequency shifts among six abundant cohorts. I obtained an estimate differing by three to four orders with the current census population size, which is most likely explained by high variance in reproductive success among individuals, genetic structure, and demographic perturbations such as historical fishing. This finding stresses the need to clearly defined conservation goals for rockfishes and marine populations in general. Fourth, I analyze contemporary samples and archived otolith samples for the heavily exploited canary rockfish to test for long-term temporal genetic changes. Results suggest that temporal differences over years clearly surpassed spatial differences among contemporary and archived sampling locations, highlighting important effects of genetic drift on this species. Lastly, I employed simulations, spatial data and N_e estimates to rapidly appraise which rockfishes within the *Sebastes* complex are more or less critical to be managed as spatially separated management units based on the relationship between overall genetic distance (F_{ST}) and population assignment (PA) of individuals to their site of origin. Using simulations and some arbitrary criteria, I conclude that it is unrealistic to obtain meaningful PA when $F_{ST} < 0.01$; therefore, species with $F_{ST} > 0.01$ should be more susceptible to lose genetic diversity if local populations are extirpated.

Spatial and Temporal Scales of Genetic Change in Two Overfished Rockfishes

by
Daniel Gomez-Uchida

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Daniel Gomez-Uchida, Author

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Finally, I am thankful to my wife Celia for her love and unconditional support during these years. She fuels my everyday life and gives sense to this journey, at times uncertain.

CONTRIBUTION OF AUTHORS

William R. Ardren and Eric A. Hoffman, formerly at the Department of Zoology (OSU), assisted during the development of microsatellite markers for canary rockfish. Most manuscripts are co-authored by Michael A. Banks, who is my major professor and has been always involved during analyses and writing of the chapters.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

Microsatellite analyses of spatial genetic structure in darkblotched rockfish (*Sebastes crameri*): is pooling samples safe?

Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*

Temporal genetic changes exceed spatial differentiation in the heavily exploited canary rockfish *Sebastes pinniger*

LIST OF FIGURES (Continued)

Figure Page

Integrating Temporal and Spatial Scales in Rockfish Population Genetics: Shaping Conservation and Management Goals

Figure

LIST OF TABLES

Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species

Microsatellite analyses of spatial genetic structure in darkblotched rockfish (*Sebastes crameri*): is pooling samples safe?

Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*

Temporal genetic changes exceed spatial differentiation in the heavily exploited canary rockfish *Sebastes pinniger*

LIST OF TABLES (Continued)

Table Page

Integrating Temporal and Spatial Scales in Rockfish Population Genetics: Shaping Conservation and Management Goals

Table

General Introduction

Pacific rockfishes (genus *Sebastes*) have attracted considerable scientific and public interest from evolutionary, fishery and conservation standpoints. Firstly, they represent a classical example of explosive radiation with over 100 species worldwide, of which 96 inhabit exclusively the northeastern Pacific and Gulf of California (Parker et al. 2000; Love et al. 2002). Secondly, the so-called *Sebastes* complex has supported a large bottom fishery along the west coast since the early 1940s (Methot and Piner 2002), and has been the subject of a long tradition of fishery research aimed at quantifying stock sizes and optimal rates of exploitation. Thirdly, heavy industrialization of the fishing industry (particularly trawling) and increased harvest effort have greatly reduced the biomass of some of the most vulnerable species to unsustainable levels (Parker et al. 2000), motivating a growing demand for recovery and rebuilding plans for these species.

Rapid advances in molecular technology have revolutionized the way evolutionary processes are studied in rockfish and other marine populations. Genetic information is being amassed and disseminated to the public at astonishing rates. Microsatellite DNA genotyping has been one of the molecular tools responsible for this progress owing to its versatility and better capabilities than other markers (Jarne and Lagoda 1996; Mueller and Wolfenbarger 1999). In the first manuscript, I present in detail the isolation and characterization of nine microsatellite DNA markers (or loci) in canary rockfish (*Sebastes pinniger*). They also work in at least 13 other

congeneric species, thus representing a valuable source of polymorphic markers for evolutionary studies within the entire group.

One key evolutionary question in rockfish biology is whether their incredible interspecific diversity is paralleled by similar amounts of variation among populations within species. However, often long larval periods among rockfishes should ensure effective dispersal (Moser and Boehlert 1991) and mixing of gene pools, thus preventing genetic differentiation. Measures of marine dispersal have therefore become pivotal to establish appropriate spatial scales of management and delimit stocks (Avise 1998), and to determine whether propagules will be exported or retained within marine reserves (Palumbi 2003). In the second and fourth manuscripts I present genetic assessments on spatial scales of dispersal for darkblotched (*Sebastes crameri*) and canary rockfish using statistical analysis of microsatellite DNA variation. In the second chapter I additionally investigate the effects of small sample sizes upon estimates of genetic parameters. For a number of reasons presented in the manuscript, equal and large sample sizes are often unavailable for offshore rockfishes. Here I devised a quantitative criterion to pool adjacent samples to enlarge sample sizes.

With the rapid depletion of fish stocks (Pauly et al. 1998), biologists are becoming more interested in describing long-term effects of harvesting. Historical analyses of fishery data have given useful insights on how systematic exploitation primarily affects abundance in predatory fish (Myers and Worm 2003). Likewise, genetic analyses of historical archived structures used in age determination (e.g., otoliths or scales) have shown loss of genetic variation owing to intense fishing

(Hauser et al. 2002; Hoarau et al. 2003). Canary rockfish is one of the most fished species in the rockfish complex—biomass estimates were at 8% of the virgin stock during 2001 (Methot and Piner 2002). In the fourth manuscript, I present microsatellite DNA analyses from an archived collection of *S. pinniger* otoliths in order to test for (*i*) significant temporal genetic changes over time, and (*ii*) linear loss of genetic variation.

Overexploitation of long-lived rockfishes has motivated fishery scientists to closely reevaluate many of the biological assumptions in fishing (Berkeley et al. 2004), and implement recovery plans (Rogers 2003). One of these considerations is based on the assessment of effective population size (N_e), a genetic parameter of great evolutionary and conservation interest (Waples 2005). A small N_e is commonly associated with high extinction risk and inbreeding depression in natural populations (Frankham 1995). Recent marine studies are strongly suggesting that, despite millions of individual fish present in a population, only a fraction (usually thousands) contribute to the next generation (Hauser et al. 2002; Turner et al. 2002. In the third and fourth manuscripts, I present estimates of N_e for darkblotched and canary rockfish by using a combination of demographic and genetic data.

Lastly, the rapid accumulation of genetic structure information for the *Sebastes* complex triggered a personal interest to write the fifth manuscript on how genetic information can be used to prioritize conservation goals within the group; particularly, which species face greater or lesser risk of losing genetic diversity if local populations are extirpated. This paper was motivated by past discussions on the management value of genetic information (Waples 1998) and the use of highly

polymorphic microsatellite DNA, which tends to "inflate" statistical significance (Hedrick 1999). Such significance, nonetheless, might not have biological meaning (Waples 1998). Here I integrate spatial information and estimates of effective population size from darkblotched rockfish, simulations, and recent advances in population genetic (Bayesian) inference to draw my conclusions.

Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species

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Abstract

The isolation and characterization of 9 polymorphic microsatellite loci (eight tetranucleotide and one dinucleotide) from the canary rockfish *Sebastes pinniger* is described. Polymorphism at these loci revealed from 6 to 28 alleles, with expected heterozygosities ranging between 0.42 and 0.88, enabling high-resolution genetic population structure investigation for this overfished species in the northeastern Pacific. They also amplify in other 13 congeneric species, providing highly variable loci for research on other rockfishes.

The northeastern Pacific rockfish assemblage (genus *Sebastes*) has been the primary resource for a major groundfish fishery since the early 1940s (Methot & Piner 2001). This multispecific stock has been subjected to particularly high harvest rates during the last two decades, mainly by the commercial trawl fleet, resulting in clear signs of overfishing in some of the more vulnerable species (Weinberg 1994, Parker *et al*. 2000, Love *et al*. 2002). Among these, canary rockfish (*S. pinniger*) has suffered a precipitous decline with current estimates at only 8% of the virgin stock abundance (Methot & Piner 2001). Conservation and rebuilding goals are thus urgently required. These should rely not only on accurate abundance estimates, but also consider the spatial distribution of genetic variation. Wishard *et al*. (1980) drew inference from an allozyme study to describe only a single continuous stock extending along the coast off from northern Washington to southern California. Here we describe the isolation of microsatellite loci in canary rockfish. Increased average

polymorphism observed for this marker type will likely resolve whether genetic differentiation is evident within this northeastern Pacific stock.

Our microsatellite enrichment protocol followed that of Hoffman *et al.* (2003) and is described below in brief. Genomic DNA was extracted from ethanol-preserved fin-clips using a DNEasy Tissue Kit (Qiagen, Valencia, CA). Degenerate Oligonucleotide-Primed Polymerase Chain Reaction (DOP-PCR), using the K6-MW primer (Macas *et al*. 1996), was used to generate a random set of DNA fragments between 200 and 2500 bp from throughout the *S. pinniger* genome with known flanking sequences. Microsatellite enrichment of the PCR amplified genomic fragments employed a 3' biotinylated $(GATA)_8$ repeat motif bound to streptavidin-coated particles (Promega, Madison,WI). Microsatellite-enriched DNA was cloned using a TOPO TA cloning kit for sequencing (Invitrogen, Carlsbad, CA). We screened 470 clones and found that 42 (9%) appeared to contain microsatellites using the protocol of Cabe & Marshall (2001), which involves a PCR step using three primers (T7, T3 and the $GATA_8$ motif repeat). Positive clones show a distinctive smear, as opposed to a single band (negative) when electrophoresed on a 2% agarose gel. Thirty-four of these were sent to the Nevada Genomics Center (Reno, NV) for automated sequencing, and 22 contained repeat motifs.

We utilized Vector NTI software (http://www.informaxinc.com) to design primers to amplify 19 unique microsatellite loci. PCR was carried out in a MJ Research™ Peltier Thermal Cycler in 5 µl-volume reaction containing 1 µl of DNA

template (25-100 ng), 1x polymerase buffer (500 mM KCl, 100 mM Tris-HCl, 1% Triton® X-100), 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.5 μM each primer (the forward primer was fluorescently labeled) and 0.1 U of *Taq* DNA polymerase (Promega). Temperature profile consisted of an initial denaturing step at 94˚C for 3 min, 30 cycles at 94˚C for 30 s, 55-58˚C for 30 s, 72˚C for 30 s, and a final extension at 72˚C for 2 min (Table 1). Amplified fragments were electrophoresed in a MJ Research™ BaseStation using 5% polyacrylamide gels and analyzed using Cartographer™ software.

Table 1. Microsatellite loci for canary rockfish (*Sebastes pinniger*). Asterisks denote the 5' fluorescent-labeled primer. *N* is the number of individuals genotyped to estimate number of alleles (A), observed (H_O) and expected (H_E) heterozygosity. Multiplex PCR was successful for *Spi*4, *Spi*6, *Spi*10 and *Spi*12, enabling characterization of greater sample sizes. A dash in the repeat motif indicates an intervening sequence (imperfect repeat).

Locus	Primer sequences (5'-3')	Repeat motif	Annealing t°	*N*	Size range	A	H_O	H_E	GenBank Accession No.
*Spi*4	*GTCAGAGTTACATAGCGTGCCCT GCACTATGGAACTGTGATTCTGGA	$(GATA)_{11}$	58˚C	83	169-217	11	0.86	0.85	AY192599
*Spi*6	*AGTGGAAGTGAACACGTAGGTTAG CACTATGGAACTGTGATGCTGG	$(GATA)_{21}$	58˚C	108	106-210	28	0.94	0.88	AY192600
*Spi*7	*CTGTCTTTGTCACTGTAATCATAGTCA GATCTGGAGTCAGATGGATAGATG	$(GATA)_{24}$	58˚C	12	136-234	10	0.07	0.88	AY192601
*Spi*9	*CATTCTTACGCACCGATCTG GAGTTTTCTTCATCTCCTTGTATTTT	$(GATA)_5$- $(GATA)_{18}$	58˚C	32	124-186	10	0.11	0.88	AY192602
*Spi*10	*TTTGATGGCCTGAAACTGAG GTTCAAACACACAGTAGCTAAACTATC	$(GATA)_{17}$	58˚C	109	115-155	10	0.77	0.82	AY192603
*Spi*12	*GGGAGTATGAGAGAGGATCATGC CAATACGCCTCCAAGCTAGATC	$(GA)_7$	58˚C	137	77-99	6	0.40	0.39	AY192604
*Spi*14	*CCAGCAGCTTGGATAGATAGTTAG GCTGGAAATACATTACACTGTTTAGTC	$(GATA)_{10}$- $(GACA)_{10}$- $(GATA)_{12}$	55˚C	12	267-317	8	0.91	0.77	AY192605
*Spi*17	*TGTTGGTTAATTACATGCTGGA TATTCCCAGCAGCTTGGATA	$(GATA)_{11}$- $(GACA)_{12}$- $(GATA)_{11}$	55˚C	12	298-342	9	0.91	0.83	AY192606
*Spi*18	*GTACAAGAAGTTAAAAAGCAAGTTGCAG GCGTGTTTGCACTAACCTTTGT	$(GATA)_5$- $(GATA)_{10}$- $(GATA)_8$- $(GATA)_6$	55˚C	25	230-328	8	0.76	0.79	AY192607

Nine primer pairs were polymorphic and amplified consistently, eight of which were tetranucleotide repeats and one dinucleotide repeat (Table 1). Five loci were monomorphic, and the remaining 5 either gave no PCR product or amplified inconsistently despite adjusting $MgCl_2$ (1.0-2.5 mM) or dNTP (80-200 μM) concentrations, and using an annealing temperature gradient. Analysis of genetic variability was performed utilizing FSTAT (Goudet 1995, Goudet 2001). Number of alleles varied between 6 and 28, and expected heterozygosities ranged from 0.39 to 0.88 (Table 1). Significant departures from Hardy-Weinberg equilibrium (P=0.0056) after Bonferroni correction were observed for *Spi*7 and *Spi*9, probably as a result of unamplifiable (null) variants. Genotypic disequilibrium was not significant in any pairwise comparison after multiple tests adjustment, providing no evidence of linkage among any of these loci.

Canary rockfish primers were screened on 13 additional rockfish species: *S. nebulosus* (china), *S. ruberrimus* (yelloweye), *S. caurinus* (copper), *S. crameri* (darkblotched), *S. diploproa* (splitnose), *S. mystinus* (blue), *S. maliger* (quillback), *S. nigrocintus* (tiger), *S. miniatus* (vermillion), *S. melanops* (black), *S. flavidus* (yellowtail), *S. auriculatus* (brown) and *S. zacentrus* (sharpchin). PCR cocktails and conditions employed were the same as in canary rockfish. Most showed homologous polymorphic products in at least two species, with the exception of *Spi*17 that did not amplify in any other species (Table 2). These loci provide the first microsatellites isolated from *S. pinniger* and are a source of variable markers for studies on other rockfishes.

Table 2. PCR analysis of canary rockfish primers screened in 13 congeneric species. The number of fish genotyped (*N*), number of alleles (A) and their size range (in base pairs) are presented.

		*Spi*4		*Spi*6		*Spi*7		*Spi*9		*Spi*10		*Spi*12		*Spi*14		*Spi*17		*Spi*18	
	N	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size
China	9	2	169-189	5	111-151	0	-	0	-	4	119-135	4	95-107	0	-	0	-	0	-
Yelloweye	9	6	169-225	8	135-183	0	-	0	-	7	119-151	3	99-103	0	-	0	-	0	-
Copper	9	9	169-237	9	111-175	0	-	0	-	3	123-131	3	81-109	0	-	0	-	8	235-291
Darkblotched	9	8	153-221	4	103-123	0	-	0	-	6	135-167	2	75-93	1	223	0	-	0	-
Splitnose	9	4	165-221	8	139-235	0	-	0	-	6	115-135	3	73-95	0	-	0	-	0	-
Blue	9	3	169-189	6	139-179	6	148-196	7	125-185	3	103-115	1	97	0	-	0	-	0	-
Quillback	9	4	169-185	7	107-151	0	-	0	-	5	123-147	4	105-123	0	-	0	-	2	247-251
Tiger	9	5	157-177	4	119-147	0	-	0	-	7	119-143	3	73-97	4	207-219	0	-	0	-
Vermillion	9	9	173-213	3	147-163	3	152-180	3	129-157	6	115-151	2	95-97	0	-	0	-	3	283-323
Black	9	4	165-197	6	99-143	9	128-192	8	101-169	4	103-119	2	97-99	0	-	0	-	0	-
Yellowtail	2	2	165-201	2	107-111	0	-	3	101-165	4	115-131	2	95-97	0	-	0	-	0	-
Brown	2	2	173-177	2	183-187	0	-	2	149-165	3	119-127	2	79-121	0	-	0	-	0	-
Sharpchin	2	0	-	2	175-199	0	-	0	-	1	127	1	85	0	-	0	-	0	-

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Microsatellite analyses of spatial genetic structure in darkblotched rockfish (S*ebastes crameri*): is pooling samples safe?

Daniel Gomez-Uchida and Michael A. Banks

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Abstract

By pooling or removing samples of small size, we investigated how results from microsatellite analyses of spatial genetic structure in darkblotched rockfish (*Sebastes crameri*) were affected. Genotypes from 6 and 7 microsatellite loci from 1206 specimens collected between Washington and California were employed in the analyses. Sample sizes varied greatly among locations (n = 11-114). When adjacent samples of $n < 25$ were pooled using an absolute genetic distance ($F_{ST} \leq 0$), the correlation between genetic and geographic distance found in the original dataset increased nearly two-fold and overall F_{ST} [95%CI] raised from 0.001 [0.000-0.002] to 0.002 [0.001-0.003]. Removing $n < 25$ samples gave a similar result; yet, the correlation increase was smaller. Another pooling strategy based on similarity tests allowed larger sizes in composite samples ($n > 100$) and further increased the correlation, although this strategy did not raise overall F_{ST}. These results indicate that under genetic isolation by distance, excessive pooling might not enhance the overall genetic differentiation among populations. The regression slope in isolation by distance plots was robust throughout all treatments and its value suggests limited dispersal distance on this species.

Introduction

Despite the widespread utilization of microsatellites or simple sequence repeats (SSRs) in evolutionary biology (Jarne and Lagoda 1996), comparatively few studies have addressed the statistical constraints associated with these highly polymorphic markers, particularly when estimating population parameters such as the amount of genetic divergence (e.g., Ruzzante 1998; Hedrick 1999; Balloux and Goudet 2002). While numerous alleles per locus enable SSRs with exceptional statistical power to detect small genetic variation among populations, their use places great importance on representative sampling to reduce the random error or "noise" on parameter estimation (Waples 1998). This statement is particularly applicable to marine fish populations, which overall exhibit low population differentiation, explained by their migration capabilities and large population sizes (Ward et al. 1994).

To determine a minimum sample size for population genetics analyses using microsatellites, nonetheless, is not trivial and researchers tend to adopt very different criteria. Using simulations, Kalinowski (2002) recommends increasing sample sizes when divergence is small among populations, thus reducing the coefficient of variation associated with commonly employed genetic distances. Ruzzante (1998), using a genetic baseline from Atlantic cod larvae, concluded that precision associated with estimates of genetic distance is increased with 50 or more individuals depending on the number of loci, number of alleles, and range of allele sizes. Other authors have utilized allele discovery curves to determine the sample size that maximizes the number of alleles found, usually ranging from 50 to 200 individuals depending on

locus variability (e.g., Banks et al. 2000; Buonaccorsi et al. 2002). When these sample sizes are not attainable, no formal procedures exist, to our knowledge, on how to reduce the amount of random sampling error and increase the level of precision in population parameters.

The marine environment often makes the task of sampling a random representation of fishes for population genetics analyses particularly difficult. Due to rapidly changing biological (e.g., spatial abundance and aggregation) as well as logistic factors (e.g., favorable ocean conditions for fishing), equal and large samples from all sectors of a population are seldom feasible. Options to address this problem could be to either pool samples of small size or simply remove them. Examples of both procedures are found in the literature (e.g., Bentzen et al. 1996; Banks et al. 2000; Roques et al. 2002). Nevertheless, principles that warrant pooling samples are not straightforward. Genetic similarity is the intuitive criterion in most cases. On the other hand, pooling samples that apparently "look" similar—based on a limited number of individuals—could be also misleading and potentially lead to spurious conclusions.

Here, we present results from molecular, population genetics and statistical analyses in darkblotched rockfish (*Sebastes crameri*), a deepwater representative from the northeastern Pacific rockfish complex. Our central objective was to test how alternate analytical strategies such as pooling or removing samples of small size affect analyses of genetic structure in this species, with emphasis on F_{ST}, one widely utilized genetic distance (Kalinowski 2002). Although the main methodological approach to compute F_{ST} incorporates a correction for random sampling error (Weir

and Cockerham 1984), precision on this estimate from experimental studies could still be affected by small sample sizes (Waples 1998).

Annual fishing surveys by the National Marine Fisheries Service (USA) have shown great variance in sample size for darkblotched rockfish (Rogers et al. 2000; Rogers 2003), making it an ideal candidate for this study. Life history of *S. crameri* includes a dispersive larval stage; pelagic young-of-the-year juveniles are found near the surface for nearly three months in offshore waters followed by settlement into bottom muddy habitats (Love et al. 2002). In principle, this long pelagic phase suggests low genetic differentiation among *S. crameri* subpopulations. Darkblotched rockfish can live up to 105 years (Love et al. 2002), although today the oldest fishes found in the fishery are commonly 38 years old (Rogers et al. 2000). Dynamics and demography of this age-structured population are well understood (Nichol and Pikitch 1994; Rogers et al. 2000) as the species sustains a great part of the northeastern Pacific rockfish fishery.

Material and methods

Biological sampling

The National Marine Fisheries Service (USA), collected darkblotched rockfish (*Sebastes crameri*) during 2001 and 2002 annual surveys using commercial fishing vessels (trawlers). Both annual cruises covered an extensive fishing ground between Washington and California. A piece of fin (fin-clip) was cut from 1206 specimens (434 in 2001 and 772 in 2002) distributed in 33 sampling locations (Fig. 1). Fin-clips were then stored in individual labeled bags and submerged in buckets containing 95% ethanol. Relevant biological information (e.g. length, weight, sex, maturity) was additionally obtained from each fish, as well as both sagittal otoliths for age determination using the break-and-burn technique (Chilton and Beamish 1982).

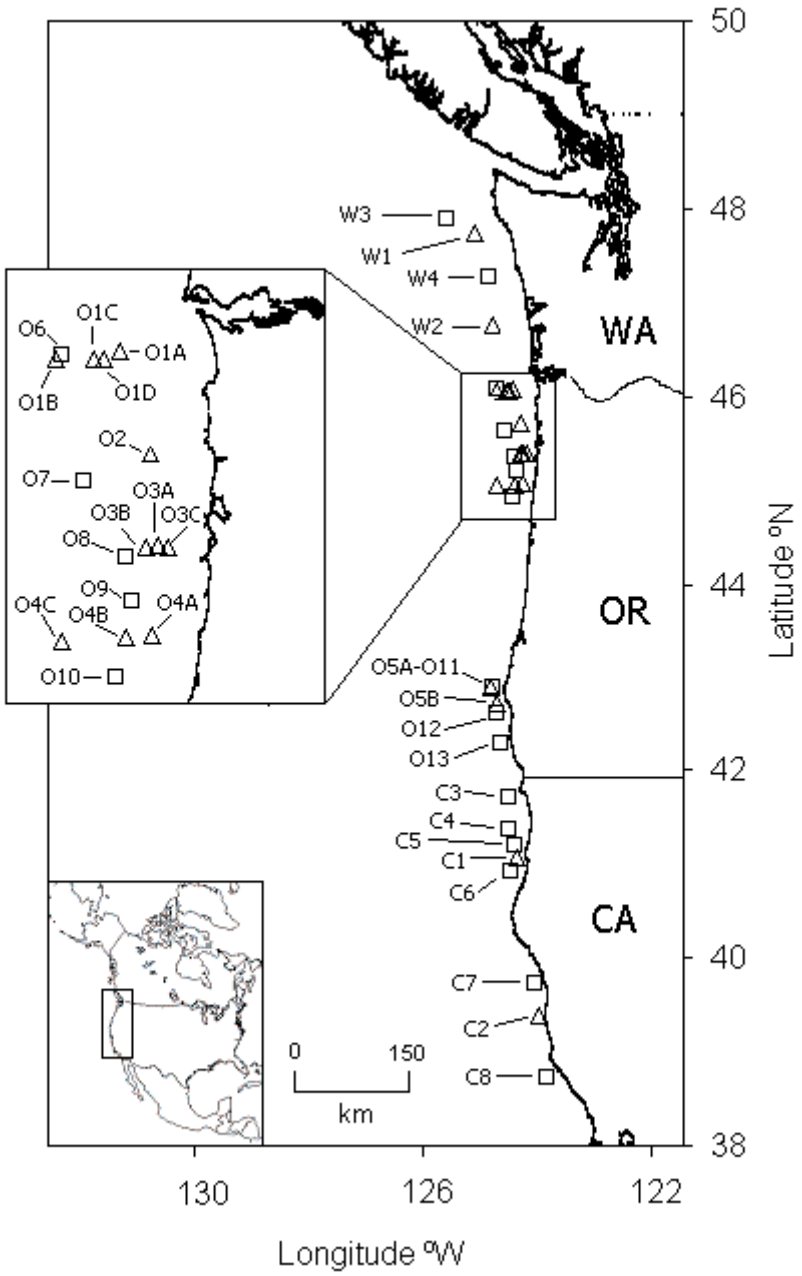

Fig.1. Distribution map of fishing sample locations during 2001 (triangles) and 2002 (squares) surveys in the northeastern Pacific (WA: Washington; OR: Oregon; CA: California).

Molecular analyses

DNA was extracted from fin-clips using a quick Chelex® (Bio-Rad) protocol as described in Banks et al. (2000). Briefly, an amount of tissue equivalent to a pinhead was put in 200 μL 5% (w/v) Chelex®, followed by a two-step temperature program (35 min at 65 ˚C, 35 min at 103 ˚C). The supernatant was used as a template in polymerase chain reaction (PCR) amplification of seven polymorphic microsatellite loci—*Sma*10, *Sma*11 (Wimberger et al. 1999), *Sal*1, *Sal*3 (Miller et al. 2000), *Spi*4, *Spi*10 and *Spi*12 (Gomez-Uchida et al. 2003). PCR reactions were carried in 5 μL volumes using authors' original cocktail concentrations for each locus. We were able to multiplex two sets of loci by adjusting primer concentrations of *Sma*10 and *Sma*11 to 0.375 and 0.5 μM respectively, and for *Spi*4, *Spi*10 and *Spi*12 using primer concentrations of 0.15, 0.5 and 0.1 μM respectively. In this last set, we had to additionally increase $MgCl_2$ from 1.5 mM to 2.5 mM for optimum amplification. Published original temperature profiles were used employing a MJ Research™ Peltier Thermal Cycler.

PCR fragments were electrophoresed in horizontal 5% polyacrylamide gels using a BaseStation™ (MJ Research™) and sized with GeneScan® 400 HD ladder (Applied Biosystems). Fragment analysis was performed in Cartographer™ software (MJ Research™), tracking amplified bands with the manual option. Genotypes were then exported to a spreadsheet for subsequent data scrutiny.

Genetic data analyses

Genetic diversity statistics

Allele frequencies and statistics of genetic variation—observed and expected average heterozygosity—were calculated in GENETIX version 3.3 (available at http://www.univ-montp2.fr/~genetix/genetix.htm). Allelic richness, an unbiased measure of allelic diversity was computed in FSTAT (Goudet 1995) following El Mousadik and Petit (1996). Markov chain exact probabilities obtained from GENEPOP version 3.3 (Raymond and Rousset 1995b) were used to test the null hypotheses of (1) Hardy-Weinberg equilibrium within samples and (2) linkage equilibrium among loci within samples. Wright's F_{IS} (Weir and Cockerham 1984) allowed testing for heterozygote deficit within samples, after randomizing alleles among individuals 5000 times and recalculating the F_{IS} value in FSTAT (Goudet 1995). Probability for the joint null hypothesis (no significant test at any locus) was calculated using Fisher's method (included in GENEPOP). This was preferred over Bonferroni's correction given its generally poor statistical power (Ryman and Jorde 2001). Departures from Hardy-Weinberg equilibrium were further investigated using MICRO-CHECKER (van Oosterhaut et al. 2004) to detect the probable source of genotyping errors.

Spatial genetic structure

Genetic structure was first investigated through Wright's F_{ST} (Weir and Cockerham 1984) in FSTAT (Goudet 1995). The statistical significance of F_{ST} over all locations and in pairwise comparisons was tested by Markov chain exact probabilities in GENEPOP (Raymond and Rousset 1995b); probability for the joint

hypothesis over all loci was calculated according to Fisher's method (e.g., Ryman and Jorde 2001). Second, we performed a hierarchical analysis of molecular variance (AMOVA) using Arlequin version 2000 (Schneider et al. 2000). Here, our goal was to separate spatial from temporal genetic changes, if found in the collection, by assessing the significance of variance components—among years (2001-2002), among samples within years and within samples.

Isolation by distance

Slatkin (1993) demonstrated that the relationship between gene flow and geographic distance for a pair of populations could be measured empirically from molecular data. Since then, this model has been applied to marine organisms to study the evolutionary implications of larval dispersal (Hellberg 1996). We used the approach developed by Rousset (1997) to study the relationship between a measure of genetic distance, $F_{ST}/(1-F_{ST})$, and geographic distance between all pairs of locations. Euclidean distances were obtained from latitude and longitude information. Because changes in longitude were negligible compared to changes in latitude in our area of study, we assumed 1º = 111 km to convert Euclidean distances into kilometers. The probability associated with the correlation was then determined using a Mantel test implemented in GENETIX.

The slope (b) from the regression between $F_{ST}/(1-F_{ST})$ and geographic distance can be used to estimate average dispersal distances, a parameter of interest in evolutionary biology, conservation and management of exploited marine populations (Palumbi 2003). One method utilizes the product $4D\sigma^2=1/b$, where D is the density of individuals and σ^2 is the variance of parental position relative to offspring position,

assuming a linear habitat (Rousset 1997). The parameter σ^2 is then related to $1/\alpha$, the average dispersal distance: $1/\alpha = 1/\sqrt{(2/\sigma^2)}$ (see Buonaccorsi et al. 2004 for details on this model's assumptions). *D* (individuals•km^{-1}) was calculated dividing an average estimate of abundance in *S. crameri* (*N* = 22 984 049) from stock assessments (Rogers et al. 2000; Rogers 2003) by the geographic scale (in kilometers) of darkblotched rockfish distribution in latitude in US waters—approximately 1110 km (between 48ºN and 38ºN, from Washington to northern California).

Clustering and multidimensional scaling (MDS)

Several suboptions of PHYLIP (Felsenstein 1993) enabled resolution of genetically related samples (clusters). Pairwise Nei's unbiased genetic distance (Nei 1987) among all samples was estimated using GENDIST. NEIGHBOR provided a summary tree using the unweighted pair-group method of arithmetic means (UPGMA). Bootstrap support on each tree node/branch was assessed applying SEQBOOT on the allele frequency data to create 1000 replicates, and CONSENSE to reach a consensus tree. All trees were visualized and edited using TreeView version 1.6.6 (Page 1996).

To graphically represent genetic closeness between every pair of samples, we utilized pairwise F_{ST} and multidimensional scaling (MDS) analysis (Kruskal and Wish 1978) implemented in the software SPSS (available at http://www.spss.com). Briefly, the MDS algorithm assigns observations (samples) to places in a two- or three-dimensional space such that the distances between observations reflect the given dissimilarity measure (genetic distance in our case).

Procedures to decrease the variance in sample size

We started pooling adjacent samples based on an absolute genetic distance—if pairwise $F_{ST} \leq 0$. F_{ST} is virtually unbiased (Weir and Cockerham 1984) and can take negative values when genetic differences are very small (Waples 1998). To avoid losing a considerable number of independent observations (i.e., degrees of freedom), only samples of $n < 25$ individuals were initially pooled. This criterion was adopted for two reasons—(1) the sample size median was around 30 and (2) the most polymorphic locus *Spi*4 showed 26 alleles (in the highly improbable case with only homozygote individuals and uniform allele frequencies, a sample with less than 26 individuals would have an underrepresented number of alleles). We compared pooling to simply removing samples of $n < 25$. In addition, a less stringent condition, based on genetic similarity from exact tests of genetic differentiation (Raymond and Rousset 1995a), allowed us to further investigate pooling combinations, aiming at sizes of 100 individuals or more for each composite sample. In one pooling combination it was necessary to exclude a sample since it differed significantly from the others. All genetic data analyses were performed on these modified datasets as well with exception of the AMOVA. For genetic isolation by distance analysis of pooled samples, we calculated an average latitude and longitude from the original samples.

Results

Original dataset

Genetic diversity statistics

Sample information and statistics of genetic variation are summarized (Table 1). Sample sizes varied greatly across locations, ranging from 11 to 114 individuals. Average observed heterozygosity fluctuated between 0.62 and 0.75; average expected heterozygosity varied between and 0.65 and 0.72. Although the total number of alleles varied between 4 (*Spi*12) and 26 (*Spi*4), mean allelic richness only ranged from 4.8 to 5.7, suggesting no large differences of allelic diversity among locations. Deviation from Hardy-Weinberg equilibrium (HWE) was present in 5 of 33 samples (W1, O1B, C3, C5 and C2). These departures were largely explained by homozygote excess in *Spi*10, as exact tests were significant only for this locus in the first four samples. Sample C2, on the other hand, only showed homozygote excess in locus *Spi*4. Results from MICRO-CHECKER suggested null alleles as the probable cause for HWE departures. Lastly, all samples were consistent with linkage equilibrium expectations (test results not shown).

Table 1. Sample collection information and genetic variation statistics from seven microsatellite loci screened in darkblotched rockfish (*Sebastes crameri*)—*n*, sample size; H_E, average expected heterozygosity; H_O, average observed heterozygosity; A_R, mean allelic richness over seven loci based on eight diploid individuals; HWE, exact probability values of fit to Hardy-Weinberg equilibrium across loci and F_{IS}, estimator of heterozygote deficit within samples. *Fisher's $P < 0.05$.

Sample code	Date	Location Lat ºN/Long ºW	*n*	Age range	H_E	H_O	A_R	HWE	F_{IS}
W3	Aug. 2002	47.90/125.59	16	5-31	0.69	0.67	5.6	0.63	0.07
W1	Aug. 2001	47.75/125.07	25	5-7	0.69	0.62	5.5	0.05*	0.12*
W4	Aug. 2002	47.27/124.85	89	2-6	0.71	0.69	5.4	0.39	0.04
W2	Aug. 2001	46.75/124.75	34	1-27	0.69	0.68	5.5	0.16	0.02
O1A	Aug. 2001	46.09/124.40	30	1-8	0.69	0.72	5.4	0.92	-0.03
O6	June 2002	46.07/124.68	11	2-3	0.69	0.69	5.4	0.71	0.05
O1B	Aug. 2001	46.06/124.70	28	2-7	0.71	0.68	5.6	0.05*	0.08
O1C	Aug. 2001	46.06/124.53	25	1-4	0.71	0.70	5.5	0.75	0.04
O1D	Aug. 2001	46.06/124.47	25	1-2	0.70	0.71	5.5	0.54	0.00
O2	July 2001	45.72/124.26	37	1-5	0.72	0.72	4.8	0.37	0.01
O7	Aug. 2002	45.62/124.57	77	3-8	0.69	0.72	5.6	0.83	-0.02
O3B	July 2001	45.39/124.27	23	1-2	0.69	0.70	5.7	0.95	0.00
O3A	July 2001	45.39/124.22	33	1-5	0.70	0.70	5.3	0.53	0.03
O3C	July 2001	45.39/124.17	16	1-5	0.67	0.69	5.4	0.76	0.01
O8	June 2002	45.35/124.37	32	2-6	0.69	0.70	5.0	0.11	0.00
O9	Aug. 2002	45.20/124.34	114	3-6	0.70	0.67	5.1	0.18	0.04
O4A	July 2001	45.06/124.59	25	1-2	0.68	0.73	5.1	0.09	-0.04
O4B	July 2001	45.06/124.37	15	2-13	0.68	0.72	5.6	0.82	-0.02
O4C	July 2001	45.05/124.68	16	4-90	0.66	0.69	5.6	0.53	-0.01
O10	Aug. 2002	44.92/124.42	56	2-6	0.71	0.73	5.5	0.29	-0.01
O11	Sept. 2002	42.89/124.78	19	3-4	0.71	0.71	5.4	0.30	0.04
O5A	July 2001	42.88/124.80	14	4-6	0.68	0.75	5.5	0.74	-0.07

O5B	July 2001	42.71/124.71	22	2-5	0.68	0.68	5.4	0.09	0.01
O12	Sept. 2002	42.61/124.71	44	3-5	0.68	0.69	5.5	0.57	0.00
O13	Sept. 2002	42.29/124.62	44	2-3	0.70	0.67	5.7	0.55	0.05
C3	July 2002	41.71/124.47	41	3-6	0.70	0.72	5.2	0.03*	-0.03
C4	July 2002	41.37/124.47	57	3-5	0.69	0.67	5.5	0.27	0.04
C5	Sept. 2002	41.19/124.37	106	2-44	0.68	0.67	5.4	0.03*	0.01
C1	June 2001	41.07/124.35	31	2-4	0.67	0.68	5.4	0.35	0.00
C6	Sept. 2002	40.93/124.44	40	3-26	0.68	0.68	5.2	0.27	0.01
C7	July 2002	39.72/124.03	15	3-6	0.66	0.66	5.4	0.50	0.03
C2	June 2001	39.39/123.95	35	2	0.69	0.67	5.3	0.03*	0.04
C8	July 2002	38.03/123.83	11	3-60	0.65	0.64	5.1	0.61	0.07
Total			1206						

Spatial genetic structure

The overall genetic differentiation was F_{ST} = 0.000, although this mainly resulted from the low polymorphism in locus *Spi*12 (F_{ST} = -0.008). When this locus was excluded, genetic differentiation was F_{ST} = 0.001 and statistically significant (Fisher's P = 0.0017). Three loci out of seven showed significant differentiation (Table 2). We also determined significant F_{ST} in pairwise comparisons among samples (Table A1).

AMOVA results revealed that the only significant variance component was found within samples (among years: %=0.06; among samples within years: %=-0.08; within samples: %=100.02), suggesting no temporal genetic changes among years.

Table 2. Comparison between original and modified datasets (after pooling or removing samples) across a range of statistics—H_O and H_E, average observed and expected heterozygosity; HWE, exact test of Hardy-Weinberg equilibrium; F_{ST}, estimate of population differentiation and 1/α, average dispersal distance. Removed or pooled samples are specified at the end; nomenclature of pooled samples followed original codes from Table 1 combined. Pooling criteria were met according to Table A1. SD = standard deviation.

Statistic	Original	Removing	Pooling by $F_{ST} \leq 0$	Pooling by exact tests[a]
Total number of samples	33	22	24	10
Mean sample size ± SD	36.9 ± 26.6	46.1 ± 27.1	50.3 ± 24.3	120.6 ± 20.2
Median	30	35	43	114
Range of H_O, H_E	0.62-0.75, 0.65-0.72	0.62-0.72, 0.67-0.71	0.64-0.72, 0.67-0.71	0.67-0.72, 0.68-0.71
HWE (only pooled samples)[b]	-	-	0.06; 0.06; 0.45; 0.29; 0.24; 0.02; 0.13	0.11; 0.47; 0.63; 0.45; 0.02; 0.19; 0.11; 0.15
Significant loci ($P < 0.05$)[c]	*Sma*10, *Sal*3, *Spi*4	*Sma*10, *Sal*1, *Sal*3, *Spi*4	*Sma*10, *Sal*1, *Sal*3, *Spi*4	*Sma*10, *Sma*11, *Sal*1, *Sal*3, *Spi*4
Overall F_{ST} [95% CI][d]	0.001**[0.000-0.002]	0.002** [0.001-0.003]	0.002** [0.001-0.003]	0.001** [0.000-0.002]
Mantel test	r = 0.163; $P = 0.036$	r = 0.201; $P = 0.041$	r = 0.247; $P = 0.015$	r = 0.421; $P = 0.037$
1/α (km)	0.87	1.22	1.10	1.00
Sample code		W3; O6; O3B; O3C; O4B; O4C; O11; O5A;	W13; O1B6; O3AC; O3B8; O4BC; O5AB11; C278	W1234; O1ABD6; O27; O3ABC8; O4ABC10;

	O5B; C7; C8	O5AB111213; C34; C12678

[a]Sample O1C was excluded.
[b]Exact probabilities are sorted in the same order as pooled samples given in the last row.
[c]Exact tests of population differentiation across locations.
[d]Excluding locus *Spi*12. 95% CI (confidence interval) was obtained by bootstrap in FSTAT (see text for details). **Fisher's $P < 0.01$.

Isolation by distance

We found a significant correlation measured by a Mantel test (r = 0.163, *P* = 0.036) between pairwise $F_{ST}/(1-F_{ST})$ and geographic distances. Using a linear density of *D* = 20 706 individuals•km^{-1} and the regression slope of Figure 2a, the average dispersal distance for darkblotched rockfish was estimated as $1/\alpha = 0.87$ km.

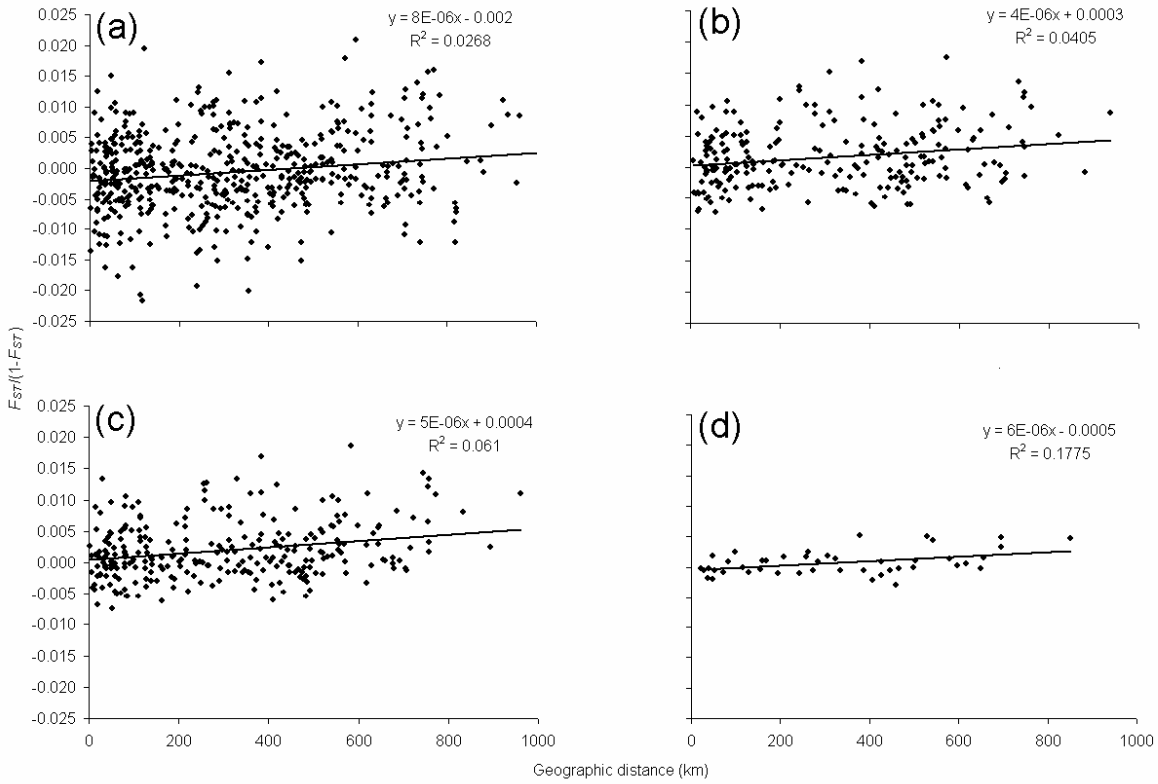

Fig. 2. Isolation by distance plots between pairwise genetic $F_{ST}/(1-F_{ST})$ and geographic distance among samples in the (a) original, (b) after removing, (c) after pooling by $F_{ST} \leq 0$ and (d) after pooling by exact tests dataset. Regression equation curves and coefficients of determination (R^2) are included.

Clustering and MDS

No major clusters were identified in the original dataset (Fig. 3a), albeit three nodes exhibited more than 50% of bootstrap support. The MDS plot (see Fig. 4a) did not show any discrete groups, although the first dimension sorted most of the samples

in a geographic fashion, with lower latitudes on the left and higher latitudes on the right.

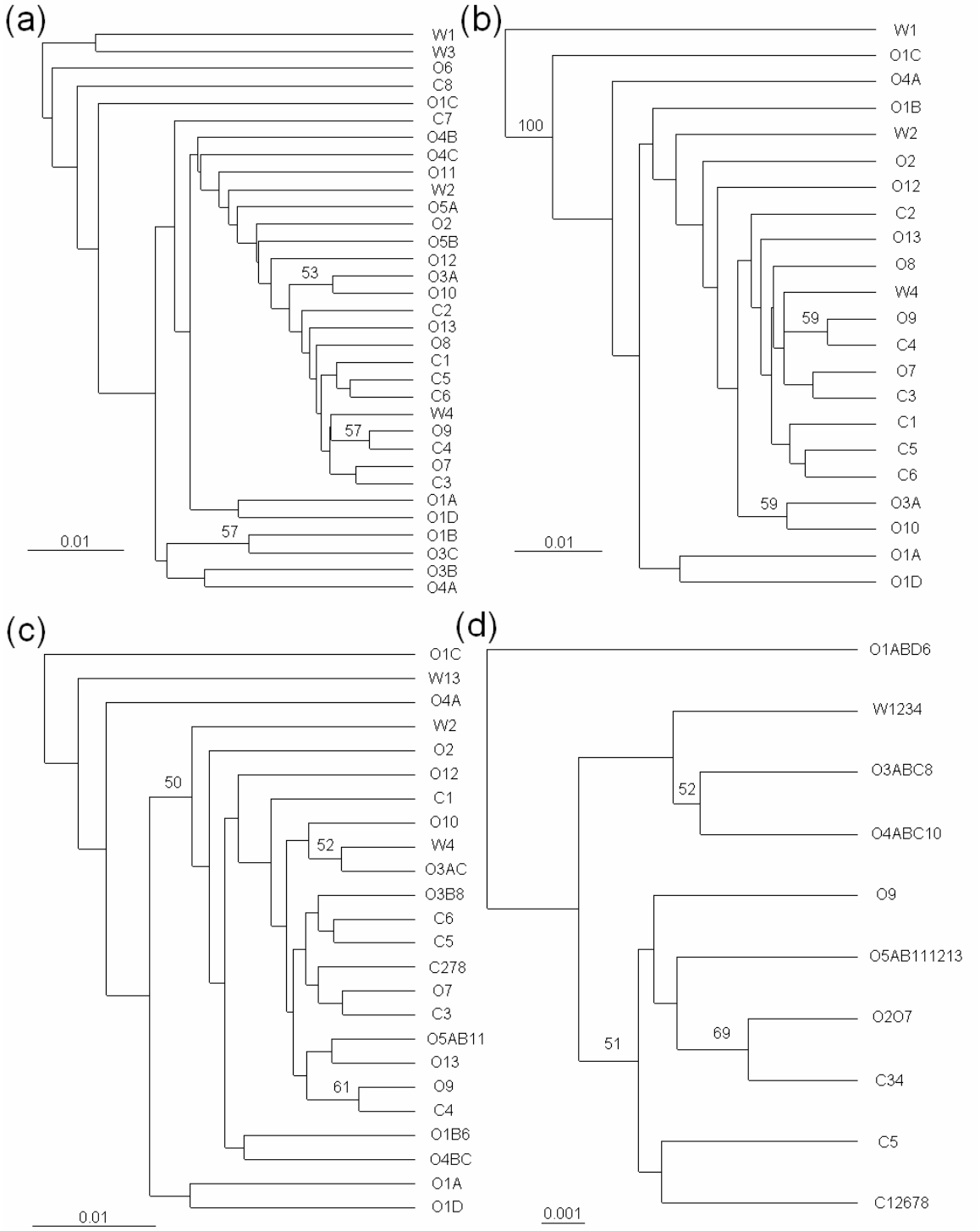

Fig. 3. UPGMA (unweighted pair-group method of arithmetic means) trees built from pairwise Nei's distances (Nei 1987) among samples in the (a) original, (b) after removing, (c) after pooling by $F_{ST} \leq 0$ and (d) after pooling by exact tests dataset. Numbers on the tree branches are percentages of bootstrap support for each node.

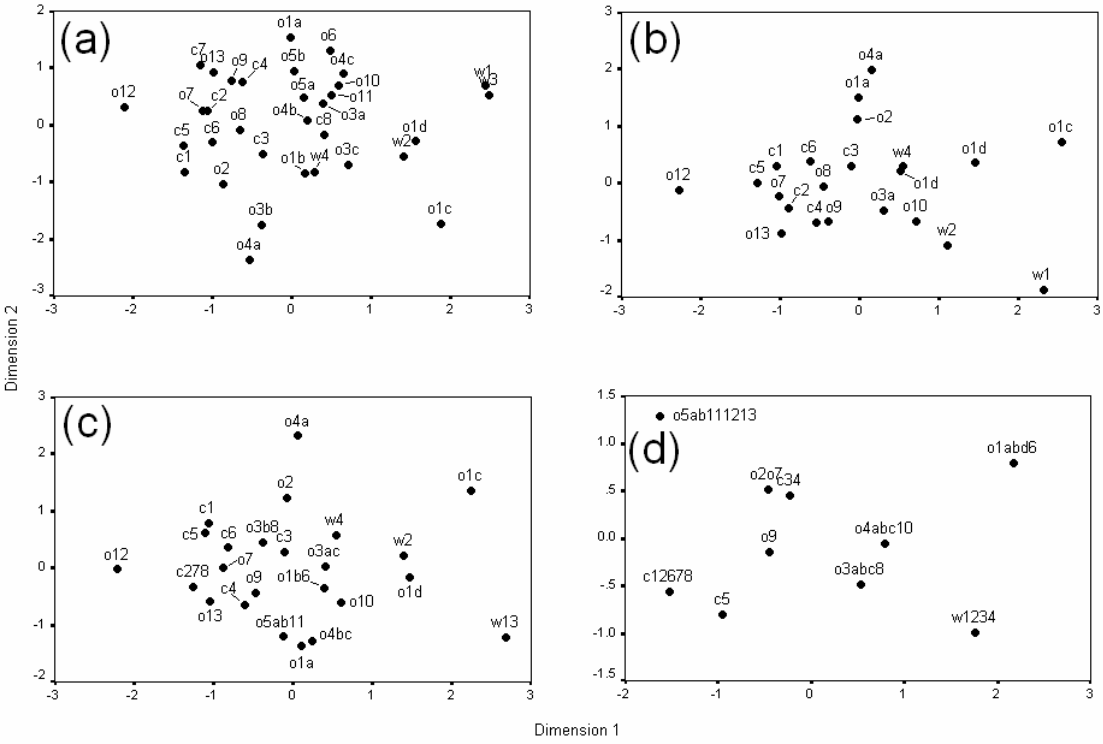

Fig. 4. Multidimensional scaling (MDS) plots from pairwise F_{ST} among samples in the (a) original, (b) after removing, (c) after pooling by $F_{ST} \leq 0$ and (d) after pooling by exact tests dataset.

Modified datasets (after removing or pooling samples)

After some samples were removed, pooled using $F_{ST} \leq 0$ or pooled using exact tests, they were compared with the original collection across a range of statistics (Table 2). Data on allelic richness was omitted from Table 2 because of differences in sample sizes.

Genetic diversity statistics

Modified datasets increased the mean and median sample size and decreased the standard deviation. Ranges of observed and expected heterozygosities became

shorter with increasing mean sample size. Deviations from Hardy-Weinberg for some of the original samples were no longer significant after pooling (W13, O1B6, C278), although others remained significant (O5AB11 and O4ABC10).

Spatial genetic structure

Removing and pooling by $F_{ST} \leq 0$ raised the overall differentiation to $F_{ST} = 0.002$, with a 95% confidence interval that did not contain zero, after excluding the low polymorphic locus *Spi*12; also, the number of significant loci increased from three to four (Table 2). However, pooling by exact tests showed the same F_{ST} value calculated for the original dataset, although the number of significant loci increased from three to five (Table 2).

Isolation by distance

Pearson's correlation (r) from Mantel tests gradually increased (Table 2), which occurred at the expense of losing considerable degrees of freedom, particularly in the last dataset (Fig. 2d). Yet, slopes from the fitted regression curves were fairly similar (Figs. 2b-d), which explains similar estimates of average dispersal distances (Table 2).

Clustering and MDS

Tree topology varied substantially across datasets and did not exhibit geographic groupings (Figs. 3b-d), even though some secondary branches showed consistency (e.g., O3A-O10 and O9-C4). MDS plots continued to sort most of the samples from south to north along the first dimension axis, with no evidence of discrete clusters (Figs. 4b-d).

Discussion

Original dataset: general patterns of population differentiation and dispersal in *S. crameri*

Our results demonstrate a statistically significant genetic structure for darkblotched rockfish between Washington and northern California, although the overall level of genetic differentiation was low and supported by only three loci (out of six utilized in the estimation). F_{ST} values found in other deepwater congeneric rockfishes such as *S. mentella* in the Atlantic (Roques et al. 2002) and *S. alutus* in the Pacific (Withler et al. 2001) are generally higher than our estimate. Cope (2004) suggests that oceanography and habitat could explain levels of genetic divergence found among rockfish populations, after comparing nearshore and offshore species. However, recent molecular and genetic analyses from grass rockfish (*S. rastrelliger*), a coastal non-migratory representative, indicate that the overall genetic differentiation (F_{ST} = 0.001) was not significant (Buonaccorsi et al. 2004). According to O'Reilly's et al. (2004) study in walleye pollock, the choice of microsatellite loci has significant influence on F_{ST} values. To which extent these estimates are thus comparable among species is still an open question.

A significant correlation between genetic and geographic distance was also found in our dataset. Isolation by distance patterns have been described in several rockfish species in the northeastern Pacific (Withler et al. 2001; Buonaccorsi et al. 2002) and northwestern Atlantic (Roques et al. 2002), indicating that gene flow is restricted to nearby populations. This implies oceanographic and/or behavioral mechanisms for subtle larval retention in *S. crameri*, despite long pelagic periods of

early development described for this species (Love et al. 2002). The mean average dispersal distance calculated from the regression slope did not exceed 1 km. These calculations rely heavily on population density estimates (Buonaccorsi et al. 2004). In our estimate of linear density (*D*), abundance of darkblotched rockfish was considered uniformly distributed, a necessary but unrealistic assumption. Palumbi (2003), on the other hand, used a dispersal function used in marine reserves (Botsford et al. 2001) to simulate mean dispersal distances that are independent of population density. Using data from Table 1 in Palumbi (2003), we fitted an exponential curve between regression slopes (*b*) and average dispersal distances (1/α') for five taxa ($1/\alpha' = 111e^{-18b}$; $R^2 = 0.98$). In the case of *S. crameri*, slopes varied between 0.004/1000 km and 0.008/1000 km across datasets, yielding $1/\alpha' \approx 111$ km. It is beyond the scope of this paper to further resolve which approach is more reliable. However, two orders of magnitude difference in the results points out the need for in-depth analysis of strengths and weaknesses of both approaches, since estimates of mean larval dispersal can have important consequences for management and conservation.

Can isolation by distance patterns be reconciled with clustering and multidimensional scaling analyses in *S. crameri*? UPGMA trees did not reveal consistent phylogenetic groups. In addition, bootstrap support for most tree branches did not exceed 50%. The MDS plots, on the other hand, showed agreement with the isolation-by-distance pattern found. Low F_{ST} values present analytical challenges in identifying discrete "stocks" in marine fishes (see an extended review of this concept in Carvalho and Hauser 1995); assessing a Wahlund's effect and/or linkage

disequilibrium in marine populations is thus difficult with microsatellites, even in hybrid zones (Nielsen et al. 2003). In other *Sebastes* species, only mitochondrial DNA sequences have identified phylogeographic boundaries in the northeastern Pacific (Rocha-Olivares and Vetter 1999; Cope 2004), stressing the differences in mutation rates and effects of genetic drift upon nuclear and mitochondrial markers.

Modified datasets: how pooling or removing small-size samples affected population genetics analyses

Our evidence indicates that pooling adjacent samples of small size ($n < 25$) using an stringent criterion enhanced values of genetic differentiation among darkblotched rockfish populations, which was reflected by a significant increment in the (1) correlation between pairwise genetic and geographic distances found in the original dataset, (2) overall measure of genetic differentiation (F_{ST}) among samples, with a 95% confidence interval excluding zero, and (3) number of significant loci from three to four. Pooling also resolved significant departures from Hardy-Weinberg equilibrium in locus *Spi*10 and *Spi4* caused by homozygote excess from putative null alleles. We did not attempt to correct for null allele frequencies using published methods (e.g., Brookfield 1996), since the main responsible locus (*Spi*10) did not contribute to genetic structuring in *S. crameri*. When small-size samples were removed instead of pooling we obtained similar results, although the correlation increment was smaller and this procedure sacrificed more degrees of freedom.

Even though pooling using exact tests made greater sample sizes and increased the isolation-by-distance correlation even further, there was no additional gain in the overall F_{ST} from the original collection, despite the increase in number of

significant loci from three to five. We noted in hindsight that sample O1C was removed from this dataset but not from the previous ones, which complicates absolute comparisons. However, we found that dropping this sample from all datasets did not produce substantial changes in the statistics reported here; most importantly, F_{ST} values remained unchanged, suggesting that pooling based on genetic similarity might create data artifacts. It has been demonstrated for microsatellite loci that F_{ST} is constrained by average expected heterozygosities; F_{ST} approaches to 0 as heterozygosity approaches to 1 (Hedrick 1999). Pooled samples of $n > 100$ in our study provided the highest heterozygosities, potentially dropping overall F_{ST} values. These findings suggest that employing a less stringent criterion to further enlarge sample sizes may not enhance differences across populations when there is genetic isolation by distance. In fish populations that do not fit this model such as Chinook salmon from California's Central Valley, pooling samples within life-history runs and correcting for family structure and admixture within samples, resolved overall F_{ST} when n=193-729 (Banks et al. 2000).

Interestingly, the slope of the regression between genetic and geographic distance did not vary substantially across datasets, implying that the model of isolation by distance is robust even if some samples comprise less than 50 individuals, challenging the general applicability of criteria to determine minimum sample sizes in marine population genetics studies.

Concluding remarks

There are two main conclusions from our study in darkblotched rockfish that could be extended to marine populations in general. First, pooling or removing

samples of small size might be sound given that they affect precision in estimates of genetic distance. Yet, removing sacrificed more degrees of freedom than pooling samples. In addition, pooling based on an absolute genetic distance ($F_{ST} \leq 0$) seems safer than the intuitive criterion based on genetic similarity and exact tests of differentiation, owing to our finding that excessive pooling might not enhance overall values of differentiation if genetic and geographic distances are correlated. Second, regression slopes from isolation by distance plots were robust to pooling and removing procedures and pointed out limited dispersal in *S. crameri*. Due to the importance of dispersal distances for conservation, localized management and restoration plans are therefore more likely to succeed.

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Appendix

Table A1. Pairwise F_{ST} (below diagonal) and exact tests of population differentiation (above diagonal) among all samples in the original dataset. *P*-values for the joint hypothesis were calculated using Fisher's method.

	W1	W2	O1A	O1B	O1C	O1D	O2	O3A	O3B	O3C	O4A	O4B
W1	-	0.293	0.004	0.184	0.22	0.028	0.006	0.181	0.172	0.539	0.013	0.376
W2	0.0036	-	0.039	0.394	0.462	0.552	0.074	0.942	0.186	0.836	0.17	0.316
O1A	0.0109	0.0105	-	0.442	0.012	0.926	0.512	0.811	0.093	0.104	0.076	0.146
O1B	0.0039	0.0025	-0.0011	-	0.362	0.513	0.45	0.703	0.661	0.955	0.292	0.694
O1C	0.0044	0.0054	0.0089	-0.0042	-	0.146	0.026	0.355	0.026	0.571	0.044	0.065
O1D	0.0071	0.0012	-0.0041	-0.0024	0.0011	-	0.263	0.842	0.048	0.842	0.188	0.19
O2	0.0129	0.004	0.004	-0.0031	0.0048	0.003	-	0.314	0.042	0.46	0.143	0.49
O3A	0.0029	-0.0068	-0.0003	-0.0016	0.0028	-0.0038	0.0019	-	0.343	0.719	0.574	0.605
O3B	0.0063	0.0028	0.005	-0.0045	0.0076	0.0068	0.0034	0.0029	-	0.77	0.643	0.268
O3C	-0.0064	-0.0062	0.003	-0.0165	-0.0047	-0.0063	-0.0026	-0.0015	-0.0105	-	0.552	0.512
O4A	0.0152	0.0062	0.0071	0.0008	0.0041	0.0052	0.0038	0.0041	-0.0027	-0.002	-	0.402
O4B	-0.0019	-0.0096	0.0012	-0.0077	0.006	0.0006	-0.0051	-0.0114	-0.0004	-0.0081	0.0028	-
O4C	-0.0037	-0.0076	-0.0028	-0.0051	0.0028	-0.0067	0.0056	-0.018	0.0091	-0.0081	0.0148	-0.0165
O5A	-0.0108	-0.004	-0.0078	-0.0077	0.0066	-0.0078	-0.0058	-0.0154	-0.0041	-0.0082	0.0088	-0.0137
O5B	0.0037	-0.0013	0.004	-0.0018	0.0107	0.0003	-0.0032	-0.0036	0.0049	-0.0065	0.0098	-0.0083
C1	0.0119	0.0046	0.0065	-0.0014	0.01	0.0075	0.0017	0.0015	-0.0007	-0.005	-0.0004	-0.0041
C2	0.0087	0.0052	0.0032	-0.0014	0.0112	0.0041	-0.0019	-0.0058	-0.0006	-0.0015	0.0043	-0.0049
W3	-0.0054	0.0029	0.0043	0.0102	0.005	-0.0031	0.011	0.0018	0.0072	-0.0003	0.0108	-0.0004
W4	0.0059	-0.0002	0.0005	-0.0053	0.0007	-0.0003	-0.0023	-0.0028	-0.0008	-0.0099	0	-0.0064
O6	-0.0016	-0.0027	0.0039	-0.0067	0.0124	-0.0031	-0.0005	-0.0118	0.0088	-0.003	0.0191	-0.0221
O7	0.0122	0.0025	0.0023	0.0001	0.0097	0.0047	0.0034	-0.0057	0.0025	0.0008	0.0049	-0.0054
O8	0.01	-0.003	-0.0004	-0.002	0.0089	-0.0007	-0.0048	-0.0071	-0.0001	-0.0016	0.0027	-0.0112
O9	0.0067	0.0033	0.0005	0.0005	0.0089	0.0051	0.0013	-0.0023	0.0041	-0.0024	0.0053	-0.0091
O10	0.0074	0.0005	0.0015	-0.0033	0.0056	0.0001	-0.0002	-0.0073	0.0039	-0.001	0.0079	-0.0051
O11	-0.0024	-0.0044	-0.0027	-0.0037	0.0008	-0.0054	0.0011	-0.0104	0.0061	-0.002	0.0092	-0.0102
O12	0.0176	0.0086	0.0076	0.0034	0.0169	0.0112	0.0041	0.0019	0.0035	0.0065	0.0086	-0.006
O13	0.0095	0.0045	0.0046	0.0016	0.0124	0.0028	0.0022	-0.0037	0.0054	0.0015	0.0089	-0.0052
C3	0.0084	0.0015	-0.0031	-0.0061	0.0038	-0.0022	-0.0005	-0.0064	0.0003	-0.0055	-0.0002	-0.0074
C4	0.0064	0.0059	0.0006	0.0017	0.0099	0.0048	0.0003	-0.0017	0.0035	-0.0027	0.0069	-0.0055
C5	0.0137	0.0028	0.0058	0.0009	0.0106	0.0059	0.0039	-0.0008	0.0021	-0.0021	0.0037	-0.0073
C6	0.0097	0.0059	-0.0017	-0.0001	0.0076	0.0029	0.001	0.0003	-0.0022	-0.0005	0.0026	-0.0062
C7	0.0069	0.008	-0.0019	0.005	0.0127	0.0113	0.0003	-0.0075	0.0085	0.0104	0.0056	0.0002
C8	-0.0053	0.0013	-0.0124	-0.0067	-0.0058	-0.009	0.0035	-0.0124	-0.0057	0.0006	-0.0034	-0.011

	O4C	O5A	O5B	C1	C2	W3	W4	O6	O7	O8	O9	O10
W1	0.663	0.769	0.045	0.00051	0.001	0.404	0.053	0.307	0.001	0.02	0.007	0.034
W2	0.876	0.632	0.379	0.015	0.0087	0.317	0.553	0.204	0.17	0.591	0.072	0.672
O1A	0.734	0.848	0.292	0.037	0.117	0.117	0.494	0.131	0.401	0.614	0.449	0.632
O1B	0.855	0.795	0.404	0.329	0.301	0.021	0.906	0.569	0.618	0.717	0.552	0.899
O1C	0.709	0.169	0.0093	0.003	0.0003	0.112	0.353	0.017	0.008	0.005	0.011	0.063
O1D	0.823	0.886	0.339	0.042	0.067	0.351	0.581	0.209	0.057	0.501	0.027	0.609
O2	0.285	0.887	0.787	0.024	0.354	0.047	0.895	0.468	0.07	0.587	0.338	0.501
O3A	0.996	0.964	0.602	0.375	0.715	0.187	0.93	0.685	0.992	0.878	0.805	0.996
O3B	0.095	0.829	0.042	0.048	0.145	0.089	0.217	0.142	0.254	0.477	0.01	0.175
O3C	0.782	0.692	0.225	0.204	0.164	0.083	0.96	0.397	0.384	0.403	0.548	0.598
O4A	0.215	0.493	0.07	0.215	0.094	0.113	0.479	0.039	0.384	0.406	0.172	0.085
O4B	0.869	0.901	0.198	0.172	0.07	0.432	0.361	0.988	0.094	0.736	0.415	0.383
O4C	-	0.841	0.718	0.357	0.463	0.698	0.939	0.851	0.696	0.351	0.886	0.986
O5A	-0.0197	-	0.772	0.46	0.88	0.289	0.92	0.978	0.961	0.978	0.716	0.948
O5B	-0.0089	-0.0127	-	0.679	0.55	0.257	0.738	0.275	0.901	0.785	0.921	0.765
C1	0.0035	-0.0045	-0.0039	-	0.243	0.0012	0.225	0.014	0.574	0.469	0.406	0.086
C2	-0.0034	-0.0132	-0.0055	-0.0033	-	0.011	0.282	0.095	0.557	0.559	0.559	0.73
W3	-0.0067	-0.0043	-0.0014	0.0158	0.0084	-	0.208	0.168	0.011	0.075	0.011	0.057
W4	-0.0034	-0.0074	0.0007	0.0003	-0.0007	0.0042	-	0.249	0.192	0.493	0.534	0.458
O6	-0.0211	-0.0205	-0.0105	0.0072	-0.0023	0.0005	0.0018	-	0.167	0.35	0.246	0.726
O7	-0.0051	-0.0106	-0.0027	-0.0017	-0.0024	0.0107	0.001	-0.0016	-	0.823	0.287	0.559
O8	-0.0041	-0.0132	-0.0043	-0.0041	-0.005	0.0071	-0.0028	-0.0061	-0.0051	-	0.617	0.693
O9	-0.0047	-0.0076	-0.0028	-0.0015	-0.0012	0.0073	0.0003	-0.0038	-0.0005	-0.0041	-	0.439
O10	-0.0085	-0.0106	-0.0024	0.0025	-0.0028	0.0037	-0.0004	-0.0075	-0.0001	-0.0025	0	-
O11	-0.0142	-0.0138	-0.011	-0.0018	-0.0032	-0.0057	-0.0019	-0.0151	-0.004	-0.0016	-0.0048	-0.0078
O12	0.0022	-0.0057	-0.0012	-0.0006	0.0003	0.0205	0.0053	0.0048	-0.0004	0.0008	0.0026	0.0099
O13	-0.0042	-0.0125	-0.0068	-0.003	-0.0037	0.0122	0.0036	-0.0085	-0.0044	-0.0035	-0.0014	-0.001
C3	-0.0066	-0.0126	-0.0048	-0.005	-0.0057	0.0078	-0.0032	-0.0058	-0.0048	-0.0059	-0.0012	-0.0015
C4	-0.0031	-0.0114	-0.0028	-0.0005	-0.0009	0.0071	0.0004	-0.0018	-0.0024	-0.0032	-0.0032	0.0007
C5	-0.0016	-0.0037	-0.0002	-0.0044	0.0003	0.0154	0.0008	-0.001	-0.0007	-0.0034	-0.0005	0.0033
C6	0.0007	-0.0084	0.0006	-0.0068	-0.0036	0.0118	-0.0009	-0.0018	-0.0019	-0.0061	-0.0016	0.002
C7	-0.0018	-0.0123	-0.0021	-0.0034	-0.0128	0.0109	0.0011	0.0012	-0.0064	-0.0061	-0.0044	-0.0027
C8	-0.0094	-0.0154	0.0024	-0.0014	-0.0056	-0.0003	-0.0024	-0.0074	-0.0033	-0.0038	-0.0045	-0.0045

	O11	O12	O13	C3	C4	C5	C6	C7	C8
W1	0.43	0.00001	0.0045	0.009	0.002	0.00001	0.039	0.11513	0.378
W2	0.743	0.00003	0.263	0.131	0.021	0.0079	0.193	0.259	0.598
O1A	0.672	0.006	0.335	0.737	0.386	0.01	0.331	0.816	0.725
O1B	0.66	0.029	0.577	0.412	0.301	0.348	0.368	0.313	0.623
O1C	0.333	0.00001	0.00259	0.0634	0.004	0.0005	0.032	0.04	0.509
O1D	0.599	0.00004	0.165	0.435	0.054	0.00516	0.146	0.224	0.668
O2	0.507	0.015	0.19	0.502	0.496	0.01	0.213	0.889	0.478
O3A	0.941	0.092	0.809	0.893	0.738	0.358	0.533	0.962	0.953
O3B	0.146	0.012	0.217	0.124	0.091	0.016	0.531	0.271	0.496
O3C	0.234	0.0034	0.338	0.724	0.333	0.203	0.213	0.043	0.073
O4A	0.095	0.00912	0.248	0.425	0.086	0.032	0.196	0.444	0.692
O4B	0.837	0.035	0.208	0.53	0.045	0.207	0.649	0.375	0.815
O4C	0.977	0.211	0.689	0.902	0.474	0.719	0.586	0.667	0.479
O5A	0.971	0.568	0.983	0.991	0.955	0.472	0.881	0.962	0.915
O5B	0.767	0.24	0.985	0.79	0.727	0.592	0.487	0.496	0.177
C1	0.367	0.164	0.533	0.586	0.092	0.741	0.802	0.289	0.249
C2	0.209	0.219	0.765	0.692	0.295	0.278	0.568	0.981	0.549
W3	0.754	0.00002	0.01	0.013	0.003	0.00003	0.018	0.134	0.305
W4	0.845	0.004	0.335	0.937	0.319	0.017	0.854	0.809	0.635
O6	0.84	0.013	0.306	0.643	0.248	0.139	0.252	0.503	0.547
O7	0.754	0.486	0.99	0.767	0.933	0.334	0.535	0.955	0.584
O8	0.381	0.07	0.752	0.484	0.594	0.444	0.874	0.883	0.614
O9	0.798	0.073	0.811	0.436	0.648	0.641	0.621	0.897	0.591
O10	0.914	0.00008	0.719	0.483	0.441	0.051	0.24	0.973	0.918
O11	-	0.114	0.905	0.418	0.599	0.316	0.639	0.93	0.843
O12	0.0001	-	0.539	0.15	0.331	0.239	0.196	0.217	0.143
O13	-0.0109	-0.0008	-	0.644	0.951	0.762	0.782	0.929	0.749
C3	-0.0039	-0.0008	-0.0026	-	0.646	0.666	0.936	0.913	0.729
C4	-0.0037	0.001	-0.0033	-0.0007	-	0.204	0.323	0.969	0.572
C5	-0.0017	0.0007	-0.0009	-0.0022	0.0008	-	0.854	0.7	0.531
C6	-0.0023	0.0011	-0.003	-0.0055	-0.0005	-0.0041	-	0.864	0.767
C7	-0.0081	0.0027	-0.0067	-0.0045	-0.0068	-0.0001	-0.0062	-	0.825
C8	-0.0123	0.0018	-0.0042	-0.0098	-0.0045	-0.0035	-0.0096	-0.0106	-

Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*

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Abstract

We report the variance effective population size (N_e) in darkblotched rockfish (*Sebastes crameri*) utilizing the temporal method for overlapping generations, which requires a combination of age-specific demography and genetic information from cohorts. Following calculations of age-specific survival and reproductive success from fishery data, we genotyped a sample (n = 1,087) comprised by six cohorts (from 1995 to 2000) across seven microsatellite loci. Our N_e estimate ($\hat{N}_e$) plus 95% confidence interval was $\hat{N}_e$ = 9,157 [6,495-12,215], showing that the breeding population number could be three to four orders of magnitude smaller than the census population size ($\hat{N}$ =24,376,210). Our estimates resemble closely those found for fishes with similar life-history, suggesting that the low $\hat{N}_e / \hat{N}$ ratio for *S. crameri* is most likely explained by a combination of high variance in reproductive success among individuals, genetic structure, and demographic perturbations such as historical fishing. These estimates highlight the vulnerability of long-lived groundfish stocks, and represent crucial pieces in resolving a complex conservation puzzle in managing ongoing harvest among rockfishes.

Introduction

Although effective population size (N_e) is a central tenet in theoretical evolutionary biology, empirical estimates of N_e have been difficult to obtain directly from natural populations, because key demographic parameters are not available throughout the entire life span of individuals (Waples 2002). The so-called temporal method overcomes this limitation by indirectly assessing the variance N_e from changes in allele frequencies between discrete generations (Waples 1989). Given that N_e represents the breeding population number and determines the intensity of genetic drift in a population (Hedrick 2000), genetic estimates have become crucial pieces in a complex puzzle to evaluate conservation risks in natural populations (Frankham 1995). For example, loss of genetic variation, inbreeding depression, and (or) greater extinction threat have been commonly associated with small N_e (Lande 1988) and low N_e / N ratios (N = census population size)(Mace and Lande 1991).

One methodological refinement of the temporal method in conservation genetics has been the combination of demographic and genetic information when generations overlap. Essentially, N_e is estimated between adjacent year-classes (cohorts) and corrected for overlapping generations using age-specific survivorship and reproductive success estimates (Jorde and Ryman 1995). This approach is suitable for ecologically and economically important long-lived marine species with multiple reproductive events (iteroparity). In addition, a long generation time complicates temporal sampling of allele frequencies among discrete generations, unless historically archived samples spanning several decades are analyzed (e.g., Hansen et al. 2002).

Here we combine age-specific demography and microsatellite DNA data from cohorts of the long-lived marine darkblotched rockfish (*Sebastes crameri*) to estimate variance effective population size. Darkblotched rockfish is one deep-water representative of the speciose genus *Sebastes* in the northeastern Pacific. *S. crameri* is characterized by long life span (maximum age recorded: 105 years)(Love et al. 2002) and late maturity (Nichol and Pikitch 1994). Low levels of biomass motivated the Pacific Fishery Management Council to declare darkblotched rockfish overfished (Rogers et al. 2000). Our goal was to provide estimates of current breeding population number and interpret their relevance to rebuilding plans (Rogers 2003) and conservation initiatives for recovery of heavily exploited rockfishes (Berkeley et al. 2004).

Materials and Methods

Samples

We obtained darkblotched rockfish samples (n = 1,206) from annual cruises by the National Marine Fisheries Service in the northeastern Pacific. A fin-clip was obtained from each specimen and preserved in ethanol for subsequent molecular analyses, along with both sagittal otoliths to determine age using the break-and-burn technique (Chilton and Beamish 1982). Once age was determined, individual fish were apportioned into cohorts or year-classes that matched calendar years.

Molecular analyses

Freshly extracted DNA from each individual was screened for seven polymorphic microsatellite loci using polymerase chain reaction (PCR), as presented in a previous study (Gomez-Uchida and Banks 2005). Extra amplification cycles (e.g.,

5-10 cycles) and an additive containing 20 mg/ml of Bovine Serum Albumin were necessary when PCR fragments were weak. Details on the electrophoresis procedures and sizing of PCR fragments are found in Gomez-Uchida and Banks (2005).

Genetic data analyses

Allele frequencies and general statistics (e.g., average number of alleles per locus, and expected and observed heterozygosity per locus) for each cohort were estimated using GENETIX version 3.3 (Belkhir et al. 2002). The null hypotheses of (1) Hardy-Weinberg equilibrium (HWE) within cohorts and (2) gametic (linkage) equilibrium among loci within cohorts were tested through exact tests implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). The probability for the joint hypothesis over multiple loci was calculated through Fisher's method implemented in GENEPOP. Departures from HWE were further investigated with MicroChecker (van Oosterhaut et al. 2004) to exclude potential genotyping errors. Finally, selective neutrality of alleles was tested through Lewontin and Krakauer's (1973) X^2 (chi-square) statistic between adjacent year-classes (Jorde and Ryman 1996).

Demographic analyses and estimation of N_e and N

Age-specific survival rates (l_i) were estimated from annual survival (S_i) calculated for thirty-eight age classes found in stock assessments of *S. crameri* (Rogers et al. 2000, 2003). We used the expression

$$S_i = \frac{1}{m}\sum_{y}^{m}\frac{N_{i,y}}{N_{i+1,y+1}} \qquad (1)$$

where $N_{i,y}$ is the number of individuals at age class i in the year y. In our case, S_i corresponds to the arithmetic mean obtained from $m = 40$ years (1964-2003) of cumulative fishery data (Rogers et al. 2000, 2003) to account for annual variation in

mortality rates. Assuming that the survival rate of the first (newborn) age class is $l_1 = 1.0$ (Jorde and Ryman 1996), then $l_{i+1} = S_i\, l_i$.

To obtain age-specific reproductive success (p_i), we used the mean number of progeny per male (b_i^m) and female (b_i^f) for each particular age class as *proxy* estimators. These are the proportion of sexually mature rockfishes at age (Nichol and Pikitch 1994) times the mean weight at age (Rogers et al. 2000, 2003). These variables were adjusted to result in a net reproduction rate $R_0 = \Sigma\, l_i b_i = 1$ for each sex. Finally, we derived a value of reproductive success weighing both sexes equally: $p_i = l_i\,(b_i^m + b_i^f)/2$ (Jorde and Ryman 1996).

N_e was estimated for the entire stock of *S. crameri* ranging from Washington to northern California using first the temporal method for overlapping generations as described in Jorde and Ryman (1995). Briefly, we used the equation

$$\hat{N}_e = \frac{C}{2G\overline{F}'} \qquad (2)$$

where C is a correction factor obtained from l_i and p_i, G is the mean generation length and $\overline{F}'$ is the grand mean of temporal allele frequency change (among all adjacent cohorts and over all loci) derived from Pollak (1983) and corrected for sampling variance. Equations to compute $\overline{F}'$, C and G are found in Jorde and Ryman (1995). In addition, the 95% confidence interval (CI) for $\overline{F}'$ and $\hat{N}_e$ was calculated according to Waples (1989) assuming a chi-square distribution for F.

The estimated census population size ($\hat{N}$) corresponded to the harmonic mean of total numbers of fish in the population between 2001 and 2002 (Rogers et al. 2000; Rogers 2003). We selected this time interval to match when genotype sampling

occurred (Gomez-Uchida and Banks 2005), because it is not clear to which time periods genetic estimates of N_e apply when generations overlap (Waples 2005).

Results and Discussion

Only genetic information from the six largest cohorts (from 1995 to 2000) (n = 1,087) was used during the rest of the analyses (Table 1). Older cohorts (≤ 1994) were comprised by less than 20 individuals, which could introduce a bias in our estimation considering some polymorphic microsatellite loci that contained up to 26 alleles (Gomez-Uchida and Banks 2005).

Both average expected and observed heterozygosity per locus did not vary significantly among cohorts; in contrast, the average number of alleles per locus was the highest in the largest cohort from 1998. No significant deviations from HWE were found in the six cohorts, and gametic equilibrium was met in all pairwise loci comparisons within each year-class. MicroChecker did not detect genotyping errors or null alleles. Chi-square tests did not exhibit significant deviations, suggesting that changes in microsatellite allele frequencies between cohorts are the sole effect of genetic drift (Table 1).

Table 1. Single- and between-cohort genetic statistics and tests for *Sebastes crameri*. Single-cohort statistics are given for one year-class (first of the pair) with the exception of the last column. H_E, expected heterozygosity; H_O, observed heterozygosity; *A*, mean number of alleles per locus; HWE, exact test for deviations from Hardy-Weinberg equilibrium. Between-cohort values include: *F'*, allele frequency change corrected for sampling error; and *P* (X^2), probability against the null hypothesis of selective neutrality of alleles.

Cohort pair	1995-1996	1996-1997	1997-1998	1998-1999	1999-2000
Sample size	48-	82-	123-	67-	627-140
H_E	0.69-	0.70-	0.69-	0.70-	0.71-0.71
H_O	0.69-	0.68-	0.68-	0.70-	0.71-0.70
A	9.4-	10.3-	10.1-	10.0-	13.0-10.6
HWE	0.36-	0.08-	0.10-	0.46-	0.68-0.11
F'	0.0032	-0.0001	0.0014	-0.0017	0.0005
$P(X^2)$	0.64	0.14	0.52	0.35	0.30

We observed a nearly exponential decline in l_i as a function of age; on the contrary, p_i peaked at 8-12 years (Fig. 1). Both age-specific variables behaved according to theoretical expectations (e.g., Jorde and Ryman 1995). With these results we then calculated the following values: $C = 110.89$ and $G = 9.7$. An estimate of $\overline{F}'$[95% CI] = 0.00064 [0.00048-0.00090] was obtained by averaging *F'* values across cohort pairs shown in Table 1. This gave an overall point estimate plus 95% CI of $\hat{N}_e$ = 9,157 [6,495 - 12,215]. Based on $\hat{N} = 24{,}376{,}210$, the ratio $\hat{N}_e / \hat{N}$ could therefore vary between 0.001 and 0.0001 for darkblotched rockfish.

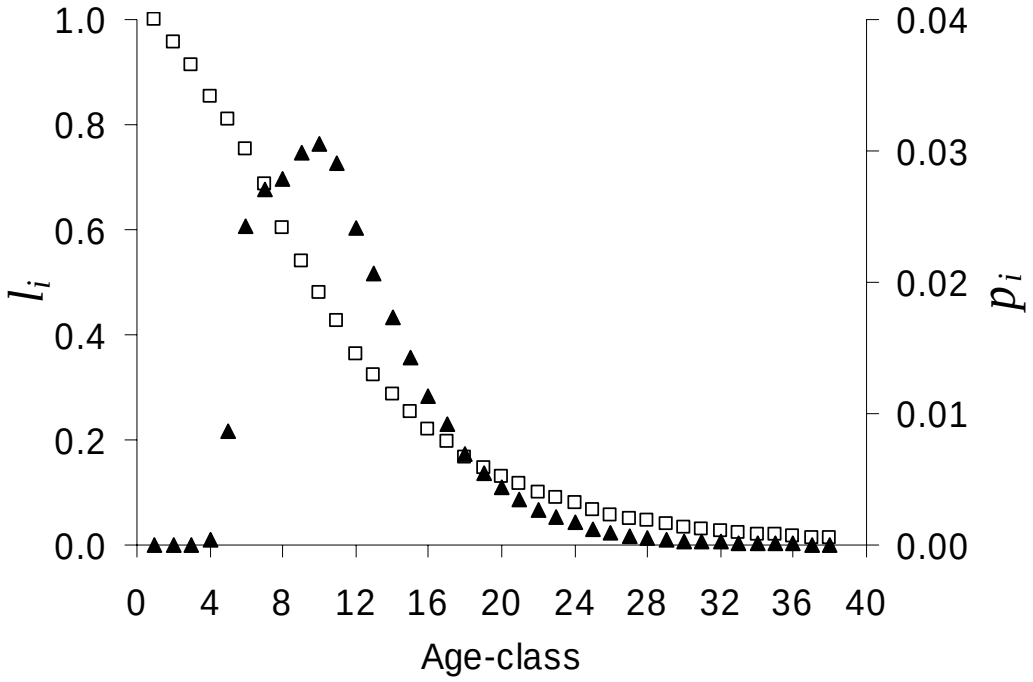

Figure 1. Survival rates (l_i) (empty squares) and rates of reproductive success (p_i) (filled triangles) as a function of age in *Sebastes crameri*.

Wright (1931) predicted that under ideal conditions—even sex ratio, non-overlapping generations, panmixia, and Poisson variance in reproductive success—the ratio N_e / N should approach unity. However, $\hat{N}_e / \hat{N}$ in natural populations average 0.1 using a variety of analytical methods (Frankham 1995), clearly indicating that these ideal conditions are commonly violated in real populations. What biological phenomena can explain the strikingly large discrepancy we observe between $\hat{N}_e$ and $\hat{N}$ values in darkblotched rockfish?

First, uneven sex ratios cannot greatly explain these differences based on extensive life-history data from *S. crameri* that indicate otherwise (Nichol and Pikitch 1994; Rogers et al. 2000; Rogers 2003). Second, overlapping generations certainly

violate ideal conditions. However, we have employed an *ad hoc* approach, with stable age-structure and constant population size being the basic assumptions of the model. Ages are implicitly assumed to have no error, although otolith reading can be difficult in older fish (Andrews 2002). Historical fishing has been demonstrated to truncate age-structure and reduce population number of numerous groundfish stocks, including our species (Berkeley et al. 2004). Jorde and Ryman (1995) argue that demographic disturbances affecting the correction factor *C* can be circumvented by monitoring the population over time. Long-term genetic data collections during the current stage of stock recovery are thus necessary to validate *C* and $\hat{N}_e$ in darkblotched rockfish.

Third, both significant spatial structure and genetic isolation by distance suggest that darkblotched rockfish is not best explained by a panmictic model, even though overall genetic differentiation was small (Gomez-Uchida & Banks 2005). In principle, a metapopulation with high stepping-stone migration, capable of exhibiting the pattern found in *S. crameri*, behaves as a large single panmictic population for purposes of estimating effective size (Wang and Caballero 1999). However, if each sub-population does not contribute equally to the metapopulation (e.g., difference in size), $\hat{N}_e$ would be underestimated (Wang and Caballero 1999) if samples from different locations are pooled as in our case. It is unlikely none-the-less that this bias alone could account for the large difference in magnitude observed between $\hat{N}_e$ and $\hat{N}$.

Fourth, our estimates seem to agree closely with published $\hat{N}_e$ and $\hat{N}_e / \hat{N}$ ratios for marine fishes with similar life-history, including red drum (Turner et

al. 2002), New Zealand snapper (Hauser et al. 2002), and Atlantic cod (Hutchinson et al. 2003). These fishes possess both high fecundity and mortality during early life stages (type III survivorship); this life strategy, when coupled with highly variable oceanographic conditions, could lead to a larger variance in reproductive success (V_k) than expected under a Poisson distribution, with the small minority of individuals ($\hat{N}_e$) replacing the entire population ($\hat{N}$) in every generation (Hedgecock 1994). Recent findings in black rockfish (*S. melanops*) indicate that older females produce larvae of better quality than younger ones, hence more capable of producing competent offspring (Berkeley et al. 2004), supporting the hypothesis that V_k could vary greatly among individual female rockfish.

In conclusion, low $\hat{N}_e / \hat{N}$ for *S. crameri* is most likely explained by a combination of factors, such as high V_k among individuals, genetic differences among sub-populations and potential demographic disturbances such as intense fishing. Our results suggest that only several thousand darkblotched rockfish rather than millions in the whole population successfully reproduce every generation, stressing the importance of sound management of vulnerable long-lived stocks (Musick 1999). However, further research is needed to (*i*) disentangle the individual effects of the aforementioned factors on N_e, and (*ii*) obtain independent estimates of key demographic parameters (e.g., Waples 2002) to validate $\hat{N}_e / \hat{N}$ ratios across marine species. Only then will we have confidence to assess long-term conservation risks of further harvest of overfished rockfishes and other marine stocks.

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Temporal genetic changes exceed spatial differentiation in the heavily exploited canary rockfish *Sebastes pinniger*

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Unpublished (to be submitted)

Abstract

Here I present microsatellite DNA analyses of contemporary samples (2001-2003) and an archived otolith collection (1973-1992) of the heavily exploited canary rockfish *Sebastes pinniger*, in order to estimate several spatial and temporal genetic parameters of interest to conservation. First, I found no overall spatial differentiation (F_{ST} = 0.001, P = 0.17), lack of genetic isolation-by-distance patterns (Mantel test: r = -0.024, P = 0.58), and no distinguishable clusters using multidimensional scaling analyses among contemporary samples screened for 10 microsatellite markers. Second, temporal differences over years (F_{ST} = 0.024, P = 0.0001) clearly surpassed spatial differences among archived sampling locations screened for two microsatellite markers, with contemporary genotypes also included in these comparisons. However, no linear trends were found for allelic richness (t = -0.851, P = 0.443) or expected heterozygosity (t = -1.060, P = 0.349) against years, even though biomass estimates declined consistently during the period of analyses. Third, allele frequency shifts between five cohorts, combined with age-specific demographic information indicate that the estimate of effective population size, $\hat{N}_e$ (95% CI) = 4694 (3464-6029), is nearly three orders of magnitude smaller than the census population size during 2001 ($\hat{N}$ = 5,453,000). These results collectively suggest that temporal genetic changes exceed spatial differences in *S. pinniger*, and allow concluding that (*i*) larval retention mechanisms proposed in other rockfishes might not have general application; (*ii*) temporal changes are likely the result of strong genetic drift, although effects of fishing on this process were not clearly established; and (*iii*) low ratio $\hat{N}_e / \hat{N}$ stresses

the vulnerability and high conservation risk of this overexploited stock in the northeastern Pacific.

Introduction

Genetic approaches in marine conservation have emerged as one important analytical tool to support management of exploited fish stocks (Avise 1998). Quintessential studies have focused mainly in resolving the question of how much demographic connectivity exists among putative populations. The answer is not simple, because the genetic "signal" of population differentiation is generally weak in marine species (Waples 1998). With the advent of new highly variable markers and more realistic models of migration (Palumbi 2003), spatial scales of dispersal and migration seem shorter than previously thought, even though life-history of early stages point to high potential for migration across long distances (e.g., Buonaccorsi et al. 2005; Gomez-Uchida and Banks 2005).

Comparative investigations addressing temporal patterns of genetic variation over years or decades have been less common, largely limited by lack of long-term sampling collections and technical limitations imposed by molecular markers (e.g., allozymes require only fresh tissue). Polymerase Chain Reaction (PCR) and microsatellite DNA genotyping technology have allowed recovering genetic information from small amounts of DNA present on fish structures in archived fishery collections (Hutchinson et al. 1999). Otoliths or scales, routinely employed in age determination, represent a look into the recent evolutionary past of harvested stocks. Recent studies have suggested the utility of historical genetic information to analyze

(*i*) stability of spatial patterns (Hutchinson et al. 2003) and (*ii*) detrimental effects of fishing (Hauser et al. 2002).

Other investigations have disclosed that effects of genetic drift (random changes in allele frequency) could be intense and might lead to inbreeding depression (Hoarau et al. 2005), a fact largely neglected in most marine populations. Effective population size (N_e) is a parameter of great evolutionary interest that measures the intensity of genetic drift and inbreeding (Frankham 1995). Despite its wide utilization to assess extinction risks for endangered species in terrestrial ecosystems (Lande 1988), similar conservation guidelines for marine ecosystems are non-existent. In an early paper, Hedgecock (1994) suggested that optimum oceanographic conditions matching reproductive events in marine populations eventually leads to successful reproduction of a limited number of individuals (N_e) that replace the majority of the population (N). Since then, numerous empirical studies have reached similar conclusions (Hedrick 2005).

Canary rockfish (*Sebastes pinniger*) is a shelf representative of the Pacific rockfish complex (Love et al. 2002 and references therein). Juvenile stages are found in shallow waters for 3-4 months (Moser and Boehlert 1991). Focused trawling effort on this species during the last two decades have reduced its biomass to record low levels (8% of the virgin stock) (Methot and Piner 2002). Life history of canary rockfish was subject to various studies during the 1980s, with emphasis in growth ecology and age determination (Boehlert and Kappenman 1980; Boehlert 1985; Boehlert et al. 1989). In this paper, I analyze the variation at microsatellite DNA markers from contemporary and archived samples of *S. pinniger* to address the

following questions: (*i*) Is spatial genetic structure homogeneous along the Pacific coast?; (*ii*) Are temporal changes in allele frequencies significant?; and (*iii*) Are temporal changes a characteristic of small effective population size?

Materials and Methods

Rockfish fin-clips and otoliths

Fin-clips ($n = 492$) were individually preserved in buckets containing 95% ethanol by staff of the National Marine Fisheries Service (NMFS) at the Northwest Fisheries Science Center (NWFSC) during 2001 and 2003 annual groundfish surveys (hereafter "contemporary" samples, collection, or data). Each fin-clip was attached to a label containing a unique barcode number that specified length, weight, sex, and age of individual fish as well as haul location (in latitude and longitude degrees) off the west coast (Fig. 1).

I additionally sampled otoliths ($n = 421$) spanning 19 years of fishery data off Oregon from an archive maintained by NMFS/NWFSC and Oregon Department of Fisheries and Wildlife in Newport (hereafter "archived" samples, collection, or data). Years sampled included 1973, 1980, 1986, and 1992. Otoliths were taken in three different ports in Oregon (Astoria, Newport and Coos Bay); however, specific geographic locations of specimens were unknown. As for fin-clips, biological data from each specimen associated with each otolith were also available.

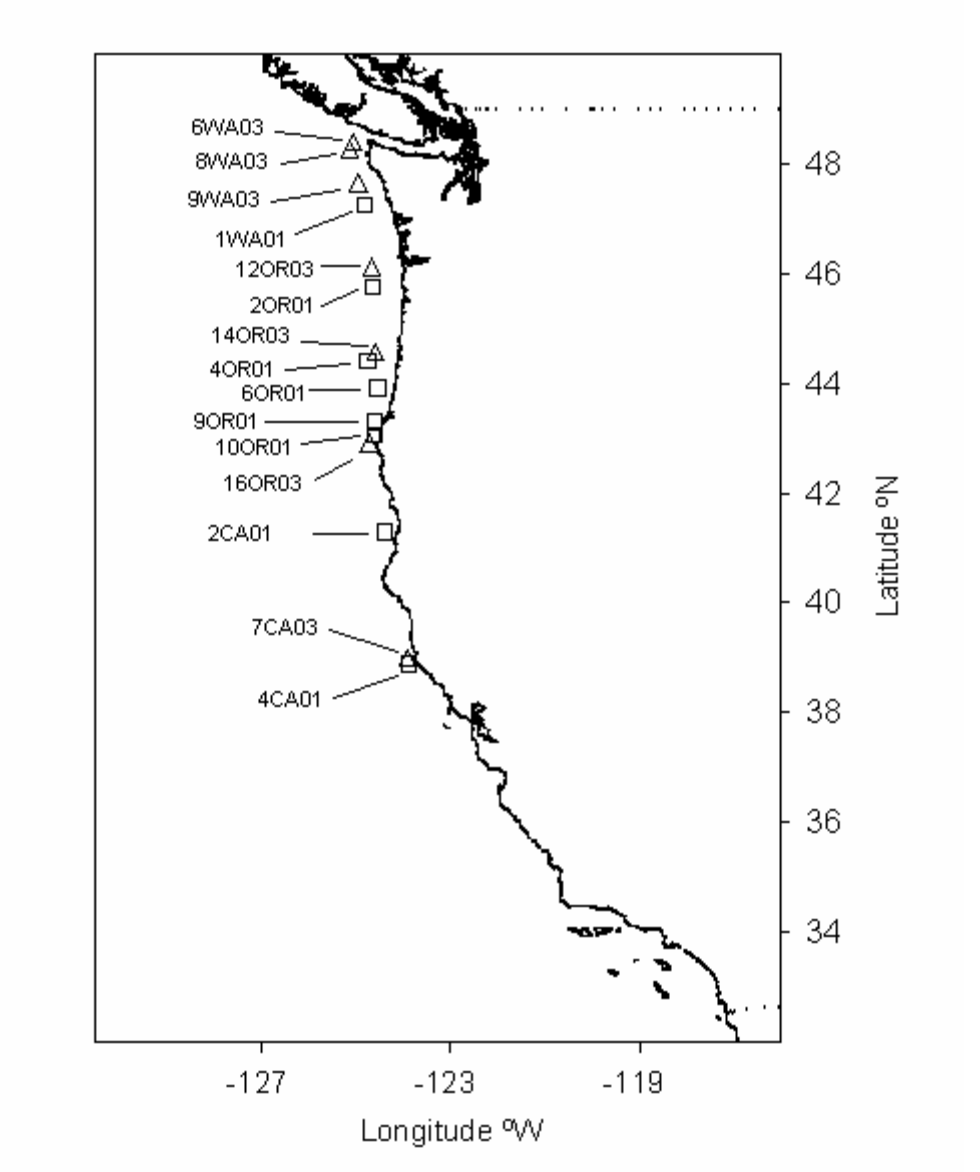

Figure 1. Distribution of sampling sites for canary rockfish (*Sebastes pinniger*) during 2001 (squares) and 2003 (triangles) annual surveys.

Laboratory procedures

DNA from fin-clips was isolated using a quick Chelex ® protocol as presented in Gomez-Uchida and Banks (2005). The extracted DNA served as template in Polymerase Chain Reaction (PCR) amplification of 10 polymorphic microsatellite loci: *Sma*3, *Sma*7 from Wimberger et al. (1999); *Sal*1 and *Sal*3 from Miller et al. (2000); *Spi*4, *Spi*12, *Spi*14, and *Spi*18 from Gomez-Uchida et al. (2003); and lastly, *Sra*7-7 and *Sra*16-5 from (Westerman et al. 2005). PCR cocktails were prepared in 5-μL volumes following original concentrations and thermal cycles for each primer plus 1 μL (20-100 ng) of template DNA. Multiplex PCR was achievable for loci *Sma*3 and *Sma*7 by simply combining both primer pairs.

To isolate DNA from otoliths, I utilized a non-destructive technique developed by Hutchinson (1999) with slight modifications. For large otoliths, I employed 15 mL Falcon^{TM} tubes during the first centrifugation step at 5500 rpm for 10 min; the supernatant was then transferred to 1.5 mL centrifuge tubes. In addition, concentration of SDS in the extraction buffer was reduced to 0.1%, which has yielded better results (W.F. Hutchinson, pers. comm. 2005). Because DNA recovered from an otolith's surface is likely to be degraded, loci selection normally includes only small microsatellite fragments (<220 bp) (Hutchinson et al. 2003). From eight markers meeting this condition, six showed inconsistent amplification peaks across a range of $MgCl_2$ concentrations. Only loci *Sma*3 and *Sal*3 showed reliable PCR products at low template DNA concentration and varying $MgCl_2$ concentrations. Final PCR cocktails in 5-μL volumes consisted of 200 μM dNTPs mix, 0.5 μM of each primer, 1X PCR ($MgCl_2$-free) buffer, 1X additive (Bovine Serum Albumin 20 mg/mL), 1.5 U of Taq

polymerase (Promega), 1 μL DNA (0.2-20 ng), and 2.5mM or 4 mM $MgCl_2$ for *Sma*3 and *Sal*3, respectively. Thermal cycling for *Sma*3 consisted of a touchdown protocol where annealing temperature was reduced from 65°C to 55°C every two cycles; nineteen additional cycles were used during the lowest annealing temperature for a total of 40 cycles. For *Sal*3, I used authors' original temperatures (Miller et al. 2000), but increased the total number of cycles from 25 to 40 cycles.

Microsatellite scoring and sizing was performed utilizing two sequencers—MJ Research™ BaseStation™, and Applied Biosytems™ ABI 3730XL™ with softwares Cartographer™ and Genemapper®, respectively. To maintain reading consistency within loci scored using both platforms, individuals representing the whole range of fragment sizes were compared between softwares and sizes were calibrated accordingly. The difference between systems was often equivalent to one tandem repeat (2-4 bp) on each locus. Scored and sized amplified fragments from each individual were verified by eye. Genotypes were then exported for statistical analyses.

Genetic variation within contemporary and archived samples

Number of alleles per locus, allele frequencies, and statistics of genetic variation within samples were estimated using GENETIX version 3.3 (Belkhir et al. 2002). Departures from Hardy-Weinberg equilibrium (HWE) and gametic (linkage) equilibrium among loci were tested through exact tests implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). Wright's F_{IS} (Weir and Cockerham 1984) was also reported to measure heterozygote deficit within samples after randomizing alleles among individuals 5000 times and recalculating the F_{IS} value in FSTAT

(Goudet 1995). I adopted Fisher's method available in GENEPOP to report probability values for the joint hypothesis over multiple loci.

Spatial genetic analyses among contemporary samples

Overall genetic differentiation was calculated through Wright's F_{ST} (Weir and Cockerham 1984) in FSTAT (Goudet 1995), and its statistical significance assessed by Markov chain exact probabilities using GENEPOP. Pairwise F_{ST} values were employed to analyze the well-documented relationship between genetic and geographic distances (Slatkin 1993), commonly described as isolation-by-distance. Estimation of genetic distances followed Rousset (1997); geographic distances were estimated according to Gomez-Uchida and Banks' (2005) guidelines for darkblotched rockfish in the same area of study. Correlation and its statistical significance between both variables were determined by means of a Mantel test implemented in GENETIX.

I additionally examined genetic closeness among locations through multidimensional scaling analysis (MDSA), an algorithm that reduces inherently multivariate data into two graphical dimensions (Kruskal and Wish 1978). Pairwise F_{ST} values served as input measures of dissimilarity in the software SPSS (available at http://www.spss.com).

Temporal changes of genetic variation among archived samples

First, I reported changes among years and locations through overall and pairwise F_{ST} values and their Markov chain exact probabilities estimated in

GENEPOP. Second, I graphically compared the allele frequency distribution throughout different years, including a contemporary sample (n = 50) from Oregon genotyped for *Sma*3 and *Sal*3. Third, a significant trend of genetic variation over time was tested through regression analysis of two unbiased measures of genetic diversity—expected heterozygosity or gene diversity (H_E) (Nei 1987) and allelic richness (El Mousadik and Petit 1996)—against years of collection as described in former studies (Hauser et al. 2002; Hutchinson et al. 2003). Average values of allelic richness and H_E for contemporary samples only using loci *Sma*3 and *Sal*3 were also included. Normality of data was verified through a Kolmogorov-Smirnov test. All analyses were performed in SPSS. Trends in *S. pinniger* estimated biomass were additionally reported as reference (Methot and Piner 2002).

Estimation of N_e and N

I estimated N_e ($\hat{N}_e$) for canary rockfish utilizing the so-called temporal method for overlapping generations on the contemporary samples. In this approach, mean standardized allele frequency changes ($\overline{F}'$; Pollak 1983) are measured between adjacent cohorts or year-classes and corrected using a factor (C) and the mean generation length (G) (Jorde and Ryman 1995): $\hat{N}_e = C / (2G\overline{F}')$. $\overline{F}'$ was obtained from spreadsheet calculations, while C and G were obtained from estimates of survival and reproductive success at age for canary rockfish. These were calculated from demographic information available in stock assessments (Methot and Piner 2002) following Jorde and Ryman's (1996) guidelines in brown trout.

Annual census size ($\hat{N}$) was estimated from canary rockfish stock assessment (Methot and Piner 2002). To estimate $\hat{N}_e / \hat{N}$ ratios, I report $\hat{N}$ as the total number of fish in the population for year 2001 (estimates for 2003 were not available).

Results

Genetic statistics within samples

While PCR was successful for most fin-clips (86%), I only amplified 42% of the otoliths sampled, thus reducing archived sample sizes extensively. Number of alleles per locus varied greatly—from 8 (*Spi*12) to 58 (*Sal*1) for a total of 175 alleles. Statistics across contemporary samples indicated no spatial trends in observed or expected heterozygosities, and HWE was met in most cases with exception of four contemporary locations and two archived samples (Table 1). Significant deviations from HWE among contemporary samples were the result of heterozygote excess in the very polymorphic *Sal*1. For archived samples, heterozygote deficit occurred in both *Sma*3 and *Sal*3. Lastly, all samples were consistent with linkage equilibrium expectations (test results not shown).

Table 1. Sample data and genetic variation statistics from microsatellite loci screened in canary rockfish (*Sebastes pinniger*)—*n*, number of genotyped individuals; H_E, average expected heterozygosity; H_O, average observed heterozygosity; HWE, exact probability values of fit to Hardy-Weinberg equilibrium across loci; F_{IS}, estimator of heterozygote deficit within samples. [1]WA: Washington; OR: Oregon; CA: California; the last two numbers correspond to the last two digits of the year of collection. [2] F = fin-clips; O = otoliths. Both tissues were screened for 14 and 2 microsatellite loci, respectively. *Fisher's $P < 0.05$. NA: Data not available.

Sample code[1]	Lat ºN/Long ºW or location	*n*	Tissue[2]	Age range	H_E	H_O	HWE	F_{IS}
6WA03	48.35/125.25	19	F	6-13	0.74	0.77	0.20	0.06
8WA03	48.15/125.34	39	F	6-15	0.75	0.76	0.01*	0.06
9WA03	47.65/125.60	20	F	6-20	0.71	0.79	0.91	-0.06
1WA01	47.15/124.80	59	F	4-22	0.72	0.73	0.26	0.04
12OR03	46.12/124.65	25	F	2-21	0.74	0.76	0.67	-0.04
2OR01	45.75/124.60	28	F	9-12	0.76	0.82	0.07	0.07
14OR03	44.54/124.40	17	F	3-13	0.70	0.75	0.54	0.14
4OR01	44.39/124.75	45	F	7-36	0.77	0.78	0.03*	0.03
6OR01	43.90/124.65	22	F	5-20	0.77	0.80	0.28	0.08*
9OR01	43.27/124.60	23	F	7-16	0.71	0.76	0.03*	0.07
10OR01	43.03/124.58	32	F	4-10	0.78	0.80	0.04*	0.04
16OR03	42.90/124.72	19	F	7-15	0.71	0.76	0.98	-0.06
2CA01	41.17/124.37	27	F	4-15	0.74	0.77	0.62	0.02
7CA03	39.10/123.79	37	F	2-11	0.75	0.77	0.87	0.02
4CA01	38.88/123.86	11	F	11-22	0.72	0.76	0.97	-0.04
CB73	Coos Bay	14	O	6-26	0.75	0.78	0.01*	0.46*

CB80	Coos Bay	15	O	15-33	0.66	0.68	0.74	0.10
NP80	Newport	42	O	7-36	0.66	0.67	0.06	0.16
NP86	Newport	50	O	6-32	0.75	0.76	0.01*	0.38*
NP92	Newport	10	O	7-42	0.56	0.64	0.32	0.28
AS92	Astoria	18	O	7-28	0.76	0.79	0.58	0.22
CB92	Coos Bay	6	O	NA	0.41	0.43	0.41	0.23

Spatial genetic structure from contemporary data

Overall genetic differentiation was small (F_{ST} = 0.001) and not statistically significant (Fisher's P = 0.17). Similarly, results from Mantel tests indicated that pairwise genetic and geographic distances were not significantly correlated (Pearson's r = -0.024, P = 0.58). Lack of geographic patterns in the data was additionally confirmed by MDSA, wherein samples from the extremes of the distribution (e.g., Washington vs. California) clustered together (Fig. 2).

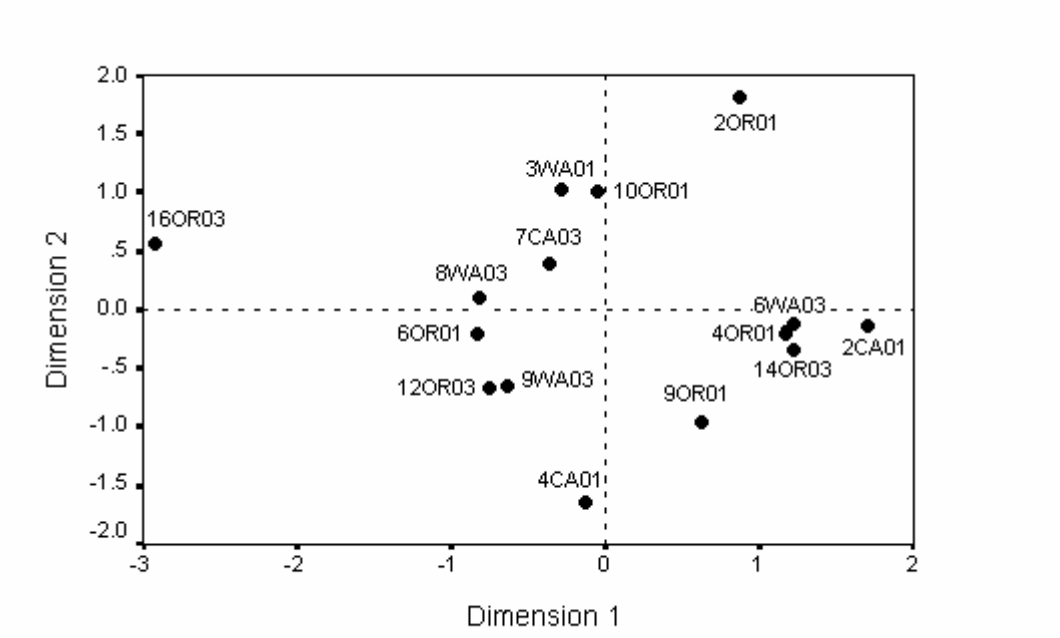

Figure 2. Multidimensional scaling analysis of parwise genetic distances (F_{ST}) among canary rockfish (*Sebastes pinniger*) samples. Sample codes are presented in Table 1.

Temporal trends in genetic variation among archived samples

Overall temporal differentiation was statistically significant (F_{ST} = 0.024, P = 0.0001). Pairwise F_{ST} values indicated stronger temporal than spatial changes—genetic differences between locations within the same year were negligible in all cases (Table 2), thus pooling genotypes across locations within years to increase archived sample sizes for each timeframe. Temporal changes in allele frequency were evident in both loci, although more marked in *Sal3*, in which some alleles are only present in specific years (Fig. 3).

Table 2. Pairwise genetic distance (F_{ST}, below diagonal) and its statistical significance measured by exact tests (above diagonal) among archived otolith samples of canary rockfish (*Sebastes pinniger*). *Significant values (P<0.05). CB, Coos Bay; NP, Newport; AS, Astoria; numbers represent the last two digits of the sample's collection year.

	CB73	CB80	NP80	NP86	NP92	AS92	CB92
CB73	-	*0.13*	*0.01*	*0.34*	*0.15*	*0.54*	*0.27*
CB80	0.02	-	*0.83*	*0.02*	*0.06*	*0.02*	*0.52*
NP80	0.03*	-0.01	-	*0.06*	*0.52*	*0.04*	*0.14*
NP86	-0.01	0.03*	0.01	-	*0.67*	*0.63*	*0.02*
NP92	-0.02	0.07	0.00	-0.04	-	*0.31*	*0.06*
AS92	-0.03	0.04*	0.02*	-0.03	-0.03	-	*0.15*
CB92	-0.01	-0.03	0.01	0.02*	0.13	0.00	-

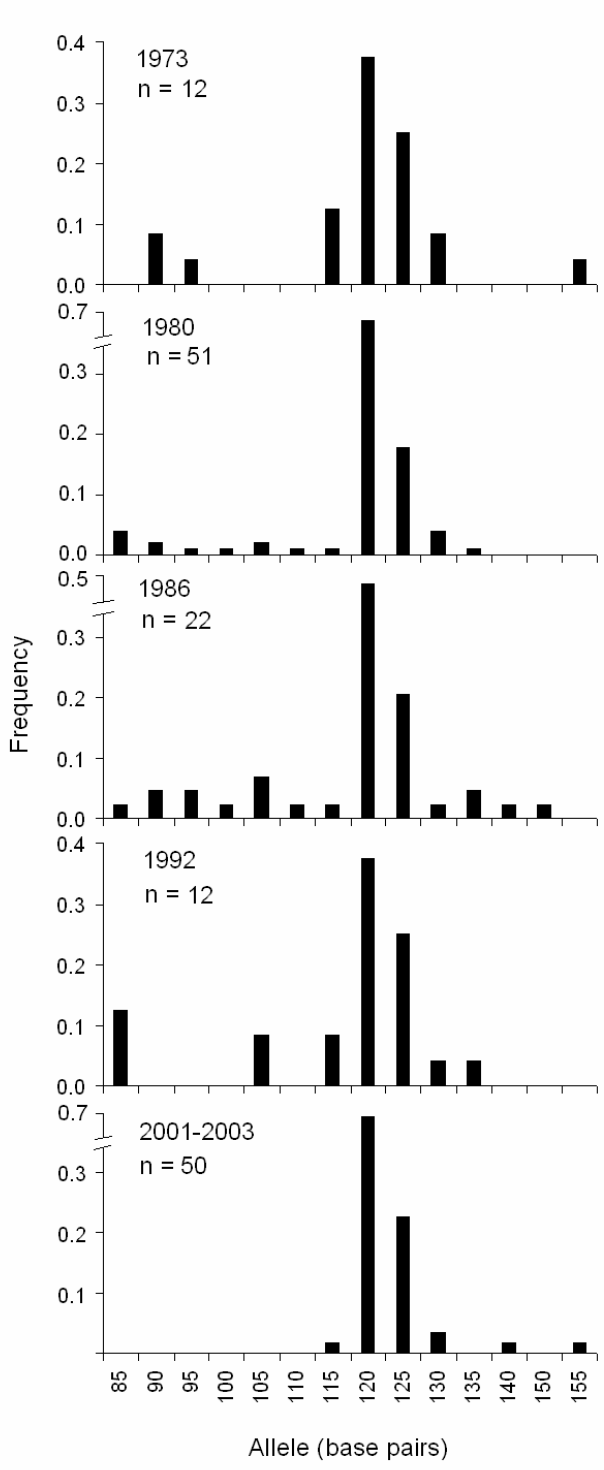

Figure 3. Temporal changes in allele frequency for locus *Sal*3 among archived and contemporary samples of canary rockfish (*Sebastes pinniger*). Sample sizes are shown below year of collection.

Temporal plots of genetic variation measures indicate that allelic richness was the highest in 1986; fluctuations in expected heterozygosity, on the other hand, were less marked despite a general decreasing tendency (Fig. 4). Kolmogorov-Smirnov tests suggested no departures from normality for both allelic richness ($P = 0.431$) and expected heterozygosity ($P = 0.665$). However, regression coefficients (slopes) for both variables were not different from zero (allelic richness: $t = -0.851$, $P = 0.443$; expected heterozygosity: $t = -1.060$, $P = 0.349$), thus suggesting no significant linear trends despite a sustained decrease in biomass estimates in the time period of our analyses (Fig. 4).

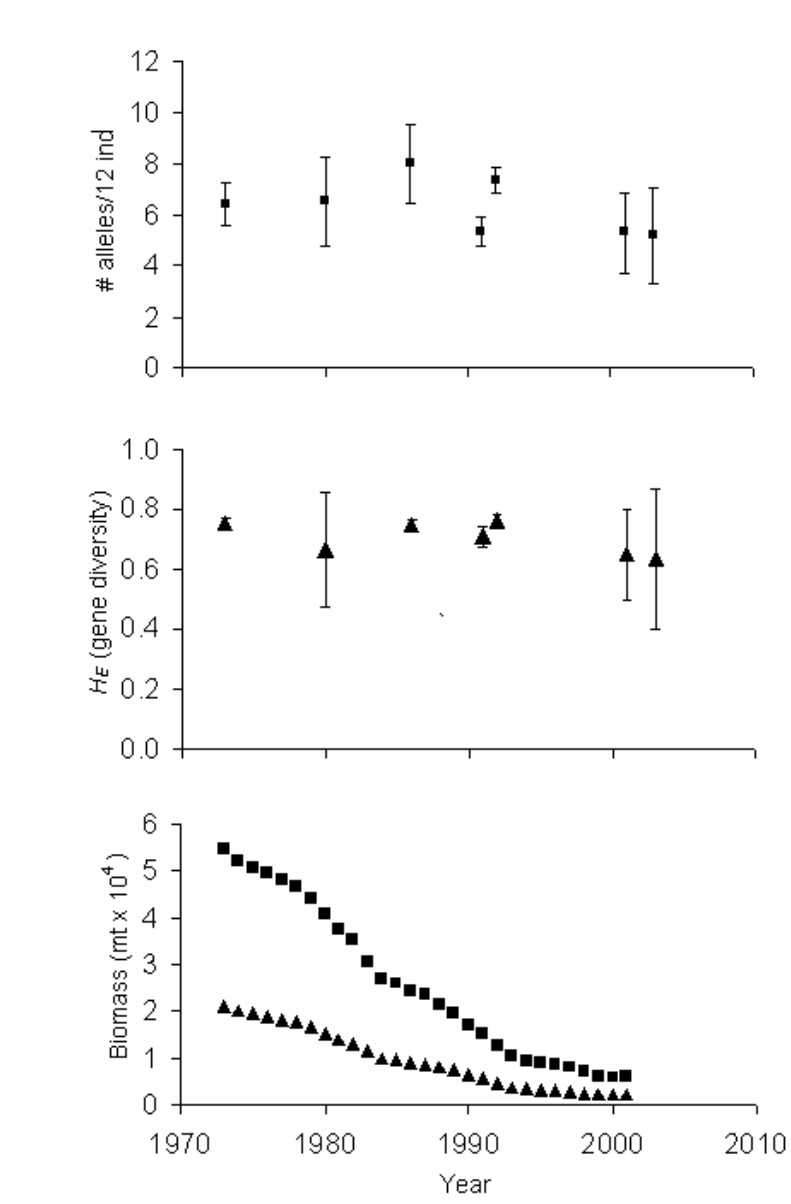

Figure 4. Temporal trends in two measures of genetic variation (top and middle) and biomass (bottom) for canary rockfish (*Sebastes pinniger*). Allelic richness is quantified as the number of alleles randomly found in 12 diploid individuals; gene diversity or unbiased expected heterozygosity (H_E) is estimated according to Nei (1987). Total (filled squares) and spawning (filled triangles) biomass estimates were taken from (Method and Piner 2002).

Estimate of N_e ($\hat{N}_e$) and ratio $\hat{N}_e / \hat{N}$

I calculated $\overline{F}'$ only from the five largest cohorts (1991-1995) (Table 3). Small sample sizes ($n < 30$) precluded the other 27 cohorts found in the contemporary collection to be utilized in the estimation. Our mean value (by averaging values from Table 3) of standardized changes in allele frequency (plus 95% CI) was $\overline{F}' = 0.0012$ (0.0009-0.0016). After including the correction factor ($C = 76.04$) and mean generation length ($G = 8.87$) in the model, I determined a $\hat{N}_e = 4694$ (3464-6029). With $\hat{N}$ (2001) = 5,453,000, I estimated a ratio $\hat{N}_e / \hat{N} \approx 0.001$ for canary rockfish.

Table 3. Cohort statistics and standardized changes in allele frequencies (*F'*) for the contemporary sample (2001-2003) of canary rockfish (*Sebastes pinniger*).

Cohort pair	1991-1992	1992-1993	1993-1994	1994-1995
Sample size	39-37	37-40	40-60	60-51
F'	-0.0033	-0.0023	0.0026	0.0067

Discussion

In this investigation, I have successfully described spatial and temporal patterns of genetic variation in the highly exploited *S. pinniger* using a combination of contemporary and archived samples. First, I found no recognizable genetic structure from Washington to California through several spatial analyses in both contemporary and archived collections. Second, I quantified significant temporal changes in allele frequencies in a span of 31 years (1973-2003), although no evidence for a linear loss of alleles or gene diversity was found. Third, I provide an estimate of effective

population size three to four orders of magnitude smaller than the current census population size. Strengths and weaknesses of these conclusions are discussed in more detail within the next sections.

Lack of spatial genetic structure from contemporary and archived data

Our analyses suggest that genetic variation in canary rockfish is homogeneously distributed within the area of study. Evidence within our contemporary collection comes from (*i*) non-significant (F_{ST}) genetic differentiation, (*ii*) lack of isolation by distance, and (*iii*) no identifiable geographic clusters in MDSA. Independent evidence for a well-mixed *S. pinniger* spawning population is found in other two sources: (*i*) a former study using allozyme variation only described weak genetic structure in identical geographic area (Wishard 1980); and (*ii*), our archived data, in which genetic differences between locations within years were negligible in all cases.

Genetic isolation-by-distance seems to be a common feature among rockfish species in the northeastern Pacific (Buonaccorsi et al. 2002, 2004, 2005; Gomez-Uchida and Banks 2005), pointing out behavioral and/or oceanographic mechanisms of larval retention along the coast, assuming that most adult benthic rockfishes are not migratory. I found no support for this hypothesis in canary rockfish, suggesting that retention mechanisms might vary within the *Sebastes* complex. For instance, bocaccio (*S. paucispinis*)(Matala et al. 2004a) and shortraker rockfish (*S. borealis*) (Matala et al. 2004b) are two other species with no significant patterns of isolation-by-distance. In general, the ecology of adult rockfishes is better understood than rockfish early life-

history, because juvenile and larval stages are difficult to identify at the species level (Love et al. 2002). Improved genetic identification methods, in addition to existing ones (e.g., Rocha-Olivares et al. 2000), will therefore play a pivotal role in studies involving rockfish larval behavior and oceanic distribution, which promises to be an active area of research clarifying the role of dispersal and oceanography on rockfish genetic structure.

Lack of identifiable geographic groups from our MDSA is a natural consequence of small genetic differentiation (Gomez-Uchida and Banks 2005; Gomez-Uchida and Banks Chapter 5). Random variation observed in Fig. 2 is likely result of sampling error and/or variance in sample sizes (Table 1). From a management perspective, separate strategies for the canary rockfish stock based on the spatial distribution of neutral genetic variation (microsatellite DNA) is therefore not warranted. Currently, the stock of *S. pinniger* is managed as a single unit off the west coast of the US (Methot and Piner 2002), thus reconciling management and genetic criteria. However, recent utilization of genetic markers under selection (e.g., Canino et al. 2005; Case et al. 2005) has opened new avenues of research that could shift the current paradigm of management by incorporating environmental variability and the distribution of adaptive genetic variation in the decision-making process.

Significant genetic changes over three decades of fishery

Despite a low rate of successful PCR from our archived collection, almost certainly as result of degraded DNA, I found that temporal changes in allele distribution were statistically significant and surpassed spatial differentiation in a period of 31 years of fishery off the Oregon coast. Hutchinson et al. (2003) described similar results in North Sea cod with marked genetic changes during 44 years of declining biomass. Likewise, Hoarau et al. (2005) uncovered unusual levels of inbreeding in a flatfish (*Pleuronectes platessa*) after a period of intense fishing after World War II. These studies point out that effects of genetic drift owing to small $\hat{N}_e$ (see following section) might be more commonplace than previously assumed in marine fish populations (Hauser et al. 2002).

However, I determined no statistical evidence for linear decrease in allelic richness and gene diversity over time, a pattern interpreted as loss of genetic diversity owing to fishing (Hauser et al. 2002). Here I present two potential explanations for this finding. First, the time span of our archived collection might be insufficient to test for loss of genetic variation; therefore, observed fluctuations in genetic variation (Fig. 3 and 4) would be the sole result of random genetic drift. Our oldest samples date back to 1973, yet a report indicates the onset of rockfish fisheries in the 1940s (Methot and Piner 2002). Samples utilized in Hauser's et al. (2002) study allowed a stronger temporal contrast since they include the fishery from unfished to heavily exploited levels. Second, fishing could potentially influence genetic variation in a non-linear fashion. For instance, Hutchinson et al. (2003) reports a decrease and increase in the total and effective number of alleles, a pattern consistent with an isolated stock

declining rapidly in size followed by subsequent influx of genetically divergent immigrants. However, this hypothesis is inconsistent with our overall finding of homogeneous genetic structure for canary rockfish.

Biological factors behind low ratio $\hat{N}_e / \hat{N}$ for canary rockfish

A theoretical population should have a ratio $N_e / N = 1$ if the following assumptions are met: panmixia, even sex ratio, discrete generations, and Poisson distribution of variance in reproductive success (V_k) (Wright 1931). Natural populations clearly diverge from these expectations after reviewing empirical estimates (Frankham 1995).

First, departures from a panmictic model probably play no important role based on our spatial analyses. Second, an uneven sex ratio in canary rockfish could potentially be a factor because males tend to outnumber females among older age classes (>12 years old) and large sizes (Methot and Piner 2002). One explanation is that older females experience higher natural mortality than males, although this statement is still controversial. It is unlikely nonetheless that a biased sex ratio solely accounts for three orders of magnitude difference between N_e and N.

Third, my context clearly violates the assumption of discrete generations because canary rockfish reproduce multiple times (Love et al. 2002). However, I account explicitly for overlapping generations in our estimate, despite some assumptions of this model do not entirely apply to our species such as stable age-structure and constant population size (Jorde and Ryman 1995). Canary rockfish exhibits truncated ages and abundance has declined steeply over time; this introduces

a degree of uncertainty on our estimate. Future genetic and demographic monitoring of the stock during its current state of recovery will verify if fishing as a demographic disturbance has affected the correction factor *C* and subsequently $\hat{N}_e$.

Fourth, an early empirical study in oysters (Hedgecock 1994) and a recent theoretical analysis (Hedrick 2005) argue that organisms with both high fecundity and mortality during early life stages (type III survivorship) have larger V_k than expected under a Poisson distribution; this could lead to a massive discrepancy between $\hat{N}_e$ and $\hat{N}$. Indeed, three- to five-order of magnitude difference have been found in ecologically similar fishes (e.g., Turner et al. 2002; Hauser et al. 2002; Hutchinson et al. 2003) and the congeneric darkblotched rockfish *Sebastes crameri* (Gomez-Uchida and Banks unpublished). Sporadic successful pulses of recruitment that vary spatially and temporally seem a general characteristic of rockfishes (Field and Ralston 2005; Love et al. 2002), and might stem from reproductive success in limited number of families. Also, Berkeley et al. (2004) found that older females of black rockfish (*S. melanops*) have a better reproductive output than younger ones, with progeny characterized by higher survival. These findings reinforce the notion that the breeding population size might be several orders of magnitude smaller than census sizes in rockfishes and other marine taxa.

In summary, the ratio $\hat{N}_e / \hat{N}$ for *S. pinniger* is most likely explained by high variance in reproductive success among individuals, and potentially a biased sex ratio. Implications of these numbers for rockfish management are important, even considering the limitations of the analytical procedures here employed (Gomez-

Uchida and Banks chapter 3). A small $\hat{N}_e$ for canary rockfish support current rebuilding initiatives and supports the concept of marked temporal genetic changes owing to strong genetic drift.

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Integrating Temporal and Spatial Scales in Rockfish Population Genetics: Shaping Conservation and Management Goals

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Biology, Assessment and Management of Pacific Rockfishes (proceedings book: submitted 09/26/05)

Alaska Sea Grant

Abstract

Genetic differentiation in space has always been interpreted as evidence for the need of separate management, although statistical differences might not reflect meaningful differences from a conservation standpoint. Here, we combine temporal estimates of effective population size (N_e) and spatial genetic structure analyses from darkblotched rockfish (*Sebastes crameri*) to simulate theoretical populations, and analyze the relationship between F_{ST} and population assignment (PA) of individuals to their site of origin. Although F_{ST} is commonly employed to measure genetic differentiation, PA also assesses the data's strength for population discrimination. We therefore aimed to define a reference F_{ST}/PA that could rapidly appraise more or less meaningful inferences to assist management of the rockfish complex. Genetic statistics for natural and theoretical populations of *S. crameri* seemed to agree for high levels of stepping-stone gene flow (>50%); however, PA was random for all individuals among three populations taken from the extremes and middle of the geographic distribution, respectively. We estimated the greatest change in slope for the F_{ST}/PA curve when F_{ST} = 0.015 and PA = 68%. In general, these results suggested that, despite significant F_{ST} values throughout all simulations, it was unrealistic to obtain meaningful PA when F_{ST} < 0.01. We discussed these findings by analyzing published F_{ST} values for many rockfishes from the northeastern Pacific, and comparing them to our reference F_{ST}/PA. Although these conclusions were drawn solely from selectively neutral markers, they provide valuable insight on which rockfish species are more or less critical to be managed as separate management units. Species in the former case should be also

more susceptible to lose genetic diversity if spatial genetic differentiation is not acknowledged.

Introduction

Analytical approaches applied in marine conservation genetics have focused almost exclusively on the spatial distribution of genetic variation. A typical study consists of comparing genotype or DNA sequence information among spatially spaced samples followed by tests against various goodness-of-fit models, often resulting in estimation of degree of connectivity or dispersal among suspected populations. On the other hand, a comparatively small number of studies have addressed genetic changes over time rather than space; we thus have little understanding of the importance of evolutionary processes among population year-classes. Demographic data and temporal genetic information combined provide an estimation of effective population size (N_e), an approach that has been featured in a growing number of recent studies (e.g., Hauser et al. 2002; Lenfant and Planes 2002; Planes and Lenfant, 2002; Turner et al. 2002; Hutchinson et al. 2003). Small N_e has been associated with a greater risk of extinction and lesser long-term population viability in natural ecosystems (Frankham 1995).

Despite the importance of both spatial and temporal genetic information, only the former has been explicitly incorporated into marine management practices (Carvalho and Hauser 1995). For instance, genetic differentiation (GD) among samples in space has been commonly understood as evidence for discrete stocks, thus warranting separate management strategies. However, Waples (1998) exposed numerous potential flaws in applying marine genetic data towards conservation

initiatives. One important conclusion from that review is the importance of identifying when statistically significant GD is biologically meaningful. Current molecular tools containing high number of alleles (e.g., microsatellites) have more intrinsic statistical power for hypothesis testing than less variable markers (Kalinowski 2002; Hedrick 1999); they are therefore more likely to show significant results, but do they reflect evolutionary processes that are biologically relevant for management?

GD is generally reported as F_{ST}, a parameter that varies theoretically between 0 and 1, although average values for marine and anadromous fish populations within species do not usually exceed 0.1 (Waples 1998). Sampling among populations with large F_{ST} results in overall (*i*) genotype frequencies that depart from Hardy-Weinberg equilibrium (HWE), and (*ii*) linkage (gametic) disequilibrium (LD) among physically unlinked loci (Hedrick 2000). Minimizing these two phenomena facilitates successful population assignment (PA) of individuals to their most likely source population by clustering methods (Pritchard et al. 2000). Therefore, PA is another direct measure of genetic data's strength to genetically discriminate populations. However, it is rarely considered when analyzing marine genetic data and their implications for management. As a rule, F_{ST} is the most commonly reported estimate in the literature (Neigel 2002).

A typical example of clear-cut management based on genetic data is found among salmon populations—a statistically significant $F_{ST} = 0.07$ among Chinook salmon (*Oncorhynchus tschawytscha*) life-history types from California's Central Valley (Banks et al. 2000) ensures PA = 99% of individuals into their natal sources (M.A. Banks, pers. comm.). In comparison, darkblotched rockfish (*Sebastes crameri*),

a typical marine example, shows $F_{ST} = 0.002$ that is also statistically significant, but clustering results in extremely low PA (<40%) owing to negligible values of LD and deviations from HWE (Gomez-Uchida and Banks 2005). This finding further pushes Waples' (1998) question, how much GD is biologically significant from a management and conservation standpoint.

Increasing numbers of endangered and overfished northeastern Pacific rockfishes (genus *Sebastes*) offer particular management challenges (Parker et al. 2000; Berkeley et al. 2004); their high ecological diversity complicates prioritization of which stocks face greater or lesser conservation risks for losing genetic diversity. In this paper, our goal was to define a reference F_{ST}/PA that enables rapid appraisal of meaningful GD for the rockfish complex. To accomplish this, we generated a series of simulated populations of *Sebastes crameri* using actual parameters from spatial genetic structure analyses (Gomez-Uchida and Banks 2005) and estimates of N_e (Gomez-Uchida and Banks, unpubl. results) in order to analyze a range of theoretical F_{ST} vs. PA values. A significant change in slope or inflexion point could be interpreted as reference. For instance, the inflexion point of the relationship between yield and fishing effort in surplus production models (i.e., maximum sustainable yield) has been a popular biological reference point utilized in fisheries assessment and management (Quinn and Deriso 1999). A similar rationale was applied during this study to estimate our reference F_{ST}/PA.

Material and methods

Previous results from population genetics analyses for darkblotched rockfish (Gomez-Uchida and Banks 2005) and an estimate of $N_e \approx 10{,}000$ from Gomez-Uchida

and Banks (unpubl. results) were utilized as input parameters to generate theoretical populations of *S. crameri*. We specified 24 populations with random mating—each containing $n = 500$ (10,000/24 ≈ 500) diploid individuals and equal number of males and females—distributed in a one-dimension stepping-stone model of migration using EASYPOP (Balloux 2001). First, we chose 24 populations based on the number of samples that maximized estimates of GD after pooling small-size samples (Gomez-Uchida and Banks 2005). Second, the stepping-stone migration model (SSMM) was utilized given the isolation-by-distance (IBD) pattern found for *S. crameri* (Gomez-Uchida and Banks 2005). IBD shows a decay of gene flow with increasing geographic distance, which is characteristic of SSMM (Slatkin 1993).

To generate a series of theoretical F_{ST} values, we run ten simulations within five migration or gene flow scenarios: 5%, 25%, 50%, 75% and 95% of individuals to adjacent demes. We chose the K-allele mutation (KAM) model (Crow & Kimura 1970) for seven unlinked loci with different mutation schemes to account for observed differences among loci in actual darkblotched rockfish data. Mutation rates were estimated according to the total number of alleles scored for each locus (Gomez-Uchida and Banks 2005) and using Ewens' (1972) relationship between the expected number of neutral alleles in a sample of $n = 250$, and $\theta = 4N_e\mu$, where μ = mutation rate (Table 8.3, Nei 1987). We then used a linear regression ($R^2 = 0.98$) to estimate θ from the observed number of alleles for each locus, and thus derive μ using N_e from Gomez-Uchida and Banks (unpubl. results). We calculated the following mutation rates: $\mu_1 = 0.000036$; $\mu_2 = 0.000042$; $\mu_3 = 0.000062$; $\mu_4 = 0.00013$; $\mu_5 = 0.00015$; $\mu_6 = 0.00015$; and $\mu_7 = 0.000010$. Populations drifted for 10,000 generations with the

option of minimal variability (i.e. all individuals start with the same allele); this setting provided a distribution of allele frequencies that best resembled those found in Gomez-Uchida and Banks (2005).

Population genotypes from each simulation were analyzed using FSTAT (Goudet 1995) for several overall genetic statistics: (*i*) level of genetic differentiation using F_{ST} (Weir and Cockerham 1984), (*ii*) LD among loci, and (*iii*) departures from HWE using F_{IT} (Weir and Cockerham 1984). We adopted the general criterion of $\alpha = 0.05$ to determine statistical significance. Results of these tests were plotted against the five migration scenarios detailed above, and a reference from actual darkblotched rockfish data for comparison. Given that each simulation is an independent event, we report average values and standard deviations to simplify results.

To estimate PA, we relied on a model-based method that probabilistically assigns individuals to hypothetical clusters so as to minimize departures from HWE and LD within each cluster (Pritchard et al. 2000). We randomly chose one simulation from each migration scenario, and chose three populations—two from the extremes (top and bottom) and one from the middle of the one-dimension stepping-stone distribution. Actual genotypes from three sampling locations described elsewhere (Gomez-Uchida and Banks 2005)—Washington (W4), central Oregon (O10) and northern California (C5)—were additionally included for comparison. Here, we utilized WHICHLOCI (Banks et al. 2003) to enlarge sample sizes to match those employed in the simulated populations ($n = 500$). We then utilized the software STRUCTURE version 2 (Pritchard et al. 2000) to determine membership coefficients for each individual into three independent clusters. The average individual

membership coefficient into each cluster provides a measure of PA. STRUCTURE settings included: burning length = 15,000 repetitions; Markov Chain Monte Carlo run = 50,000 iterations; ancestry model = admixture; and allele frequency model = correlated (Falush et al. 2003). All runs assumed K (number of populations) = 3.

We finally plotted the relationship between F_{ST} and average PA among clusters for each migration scenario, and sought inflexion points or compared the slopes of each section of the curve. Slopes within each section were estimated through $S = \Delta F_{ST}/\Delta PA$, where Δ is the difference between adjacent values.

Results

Genetic statistics

All statistics decreased in a similar fashion as a result of increasing levels of migration (Fig.1). The highest values were obtained with 5% of migration and the lowest with 75% or 95% of migration. Results from these high levels of gene flow best resembled actual darkblotched rockfish data. All F_{ST} values were statistically significant throughout all simulations.

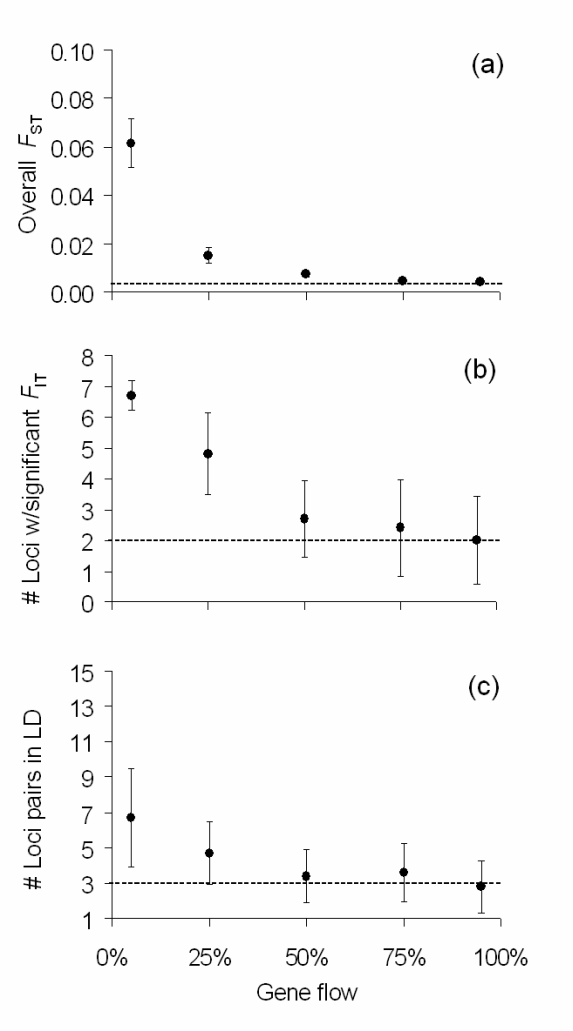

Figure 1. Plots of genetic statistics such as (a) overall F_{ST}, (b) departures from Hardy-Weinberg equilibrium (F_{IT}), and (c) linkage (gametic) disequilibrium (LD) as a function of varying levels of gene flow among theoretical populations of *Sebastes crameri*. Actual genetic statistics for *S. crameri* (Gomez-Uchida and Banks 2005) are represented with a dashed line for comparison (# = number).

PA of clusters

PA diminished rapidly as migration increased (Table 1). For the lowest level of migration (5%), PA of individuals to clusters was the highest (>95%) and cluster separation was clear with a few misclassified individuals (Fig. 2a). When gene flow was 25% and 50%, PA varied between 72% and 51%, respectively (Table 1); here, between 30% and 50% of individuals were generally misclassified (Fig. 2b and 2c).

Successful PA was not attainable for migration simulations with 75% and 95% of gene flow. Evidence for this is found in almost equal PA among clusters (Table 1) and poor cluster separation (Fig. 2d and 2e). Darkblotched rockfish PA from actual data were most similar to those found within the last two migration scenarios (Table 1, Fig. 2f).

Table 1. Population assignment (PA) of three theoretical populations—top, middle and bottom—from the one-dimensional stepping-stone distribution, and three actual samples of *Sebastes crameri* taken off Washington (W4), Oregon (O10), and California (C5) (Gomez-Uchida and Banks 2005) to three clusters. PA was obtained from average individual membership coefficients of each population or sample into each cluster using the software STRUCTURE version 2 (Pritchard et al. 2000). Migration scenarios indicate percentage of individuals exchanged to adjacent theoretical populations. Actual samples were enlarged to a size of $n = 500$ using WHICHLOCI (Banks et al. 2003) to match theoretical population sizes.

Migration scenario	Top			Middle			Bottom		
5%	1	2	3	1	2	3	1	2	3
	0.02	0.96	0.02	0.91	0.05	0.04	0.02	0.02	0.96
25%	1	2	3	1	2	3	1	2	3
	0.21	0.07	0.72	0.61	0.19	0.20	0.20	0.72	0.08
50%	1	2	3	1	2	3	1	2	3
	0.60	0.26	0.14	0.23	0.51	0.26	0.17	0.23	0.60
75%	1	2	3	1	2	3	1	2	3
	0.31	0.35	0.34	0.33	0.33	0.34	0.35	0.32	0.33
95%	1	2	3	1	2	3	1	2	3
	0.34	0.32	0.34	0.33	0.34	0.33	0.33	0.34	0.32
Actual		W4			O10			C5	
samples	1	2	3	1	2	3	1	2	3

	0.31	0.36	0.35	0.32	0.32	0.36	0.33	0.34	0.32

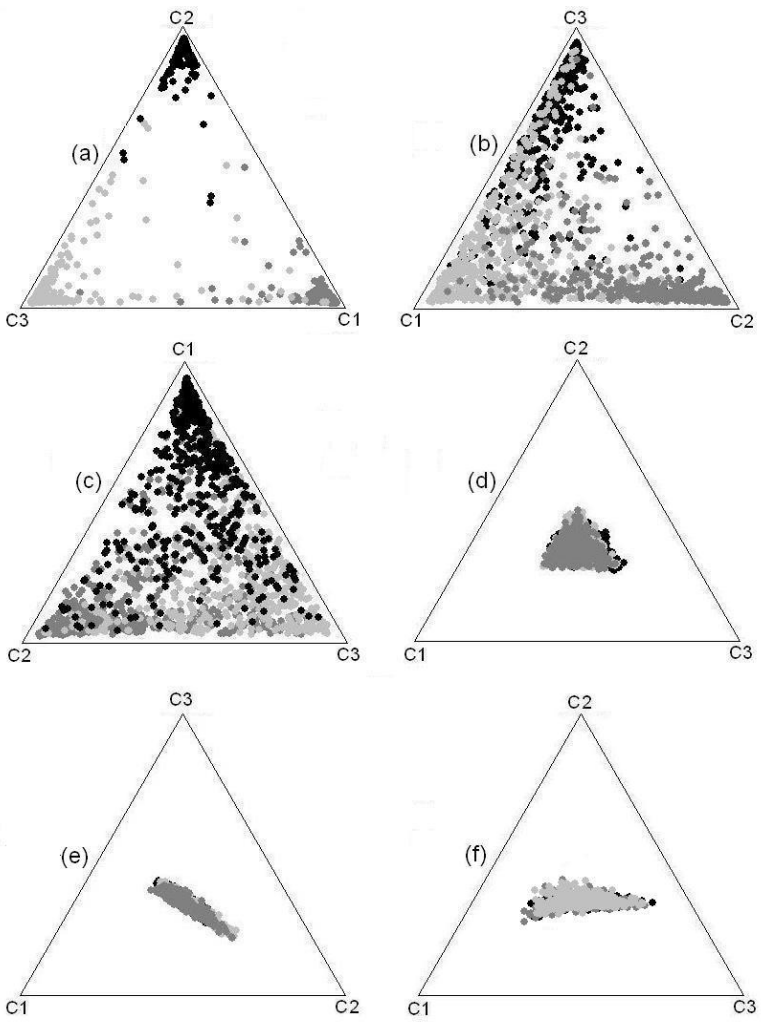

Figure 2. Diagram of population assignment (Pritchard et al. 2000) of three theoretical populations of *Sebastes crameri* (black, light grey, and dark grey) to three clusters (triangle's vertices) in simulations with (a) 5%, (b) 25%, (c) 50%, (d) 75%, and (e) 95% of stepping-stone migration. Description of the theoretical populations is found in Table 1. (f) was obtained using three actual *S. crameri* samples off Washington (W4), Oregon (O10) and California (C5)(Gomez-Uchida and Banks 2005) after enlarging sample sizes using WHICHLOCI (Banks et al. 2003) to match theoretical population sizes (n = 500).

F_{ST} vs. PA

We were not able to distinguish an inflexion point that suggests a change in the curve's shape (Fig. 3). However, calculations using the slopes within section a, b and c of the curve (S_a = 0.18, S_b = 0.07, and S_c = 0.01) indicate that (S_a - S_b) > (S_b - S_c), suggesting that the curve shows a more significant change beyond the point signaled by the arrow in Fig. 3, when F_{ST} = 0.015, and PA = 68%.

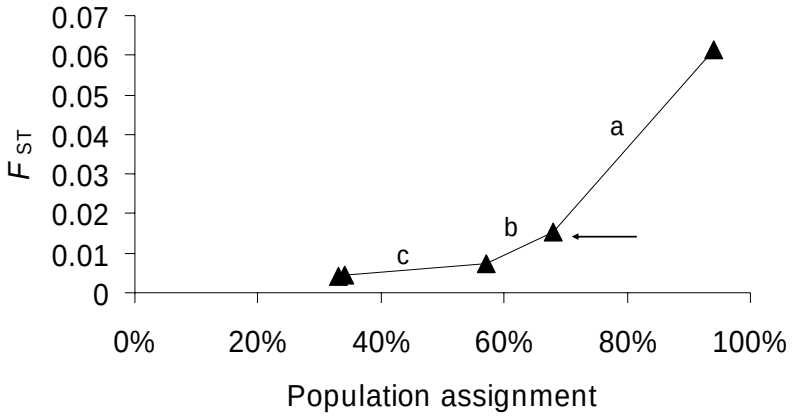

Figure 3. Plot of F_{ST} (genetic differentiation) from theoretical populations of *Sebastes crameri* vs. successful assignment of individuals to their population of origin using model-based clustering (Pritchard et al. 2000). Letters a, b, and c represent three sections of the curve. The arrow signals the point where the curve shows the greatest change in slope.

Discussion

Use of temporal N_e in spatial simulations: natural and theoretical darkblotched rockfish populations

In this paper, we combined temporal and spatial genetic information to simulate theoretical populations of darkblotched rockfish. The former was used to specify population sizes and mutation rates; the latter served to identify the most likely

migration model. Computer simulations can represent evolutionary processes under versatile conditions, and have been adopted increasingly to address complicated issues in population genetics, such as determining minimum sample sizes (Kalinowski 2005) and estimating dispersal distances for marine populations (Palumbi 2003). Our results indicate that high levels of migration (>75%) provided genetic statistics that best resembled those described for *S. crameri* in the northeastern Pacific (Gomez-Uchida and Banks 2005). This background of high gene flow to adjacent aggregations only allowed 33% of PA for three samples, not enough to identify the natal origin of individual fish. While it is difficult to exactly simulate the actual evolutionary process, these results are in agreement with the life history of darkblotched rockfish, which includes a long larval period (Love et al. 2002) that promotes dispersal between neighbor aggregations. Owing to the stepping-stone model of migration, overall genetic differentiation was still observable and statistically significant. Nonetheless, indiscriminant PA reinforces the notion that significant yet small GD does not necessarily imply meaningful results for management applications.

Reference F_{ST} for the rockfish complex

Although a clear inflexion point was not found in the plot between F_{ST} and PA, we have presented a reference F_{ST}/PA value after which the curve undergoes the highest change in slope. Also, our findings demonstrate a sustained decrease in PA for $F_{ST} < 0.01$. To determine the origin of individual fish for this level of GD is not attainable, even if they are separated by more than a thousand kilometers (e.g., Gomez-Uchida and Banks 2005). We could use this point as a reference to classify

rockfishes according to published F_{ST} values using microsatellites. For instance, boccacio *S. paucispinis* (F_{ST} = -0.001; Matala et al. 2004a), grass rockfish *S. rastrelliger* (F_{ST} = 0.001; Buonaccorsi et al. 2004), shortraker rockfish *S. borealis* (F_{ST} = 0.001; Matala et al. 2004b), canary rockfish *S. pinniger* (F_{ST} = 0.001; Gomez-Uchida and Banks chapter 4), and darkblotched rockfish *S. crameri* (F_{ST} = 0.002; Gomez-Uchida and Banks 2005) are located to the left of the reference, where spatially explicit management would be less critical; Pacific Ocean Perch *S. alutus* (F_{ST} = 0.015; Withler et al. 2001), black rockfish *S. melanops* (F_{ST} = 0.018; Miller et al. 2005), copper rockfish *S. caurinus* (F_{ST} = 0.036; Buonaccorsi et al. 2002), brown rockfish *S. auriculatus* (F_{ST} = 0.056; Buonaccorsi et al. 2005), and cryptic species of vermillion rockfish *S. miniatus* (F_{ST} = 0.097; J.R. Hyde, pers. comm.) and rougheye rockfish *S. aleutianus* (F_{ST} = 0.301; Gharret et al. 2005), on the other hand, are positioned towards the right of this reference, where spatially explicit management would be more critical.

We have reached these conclusions assuming that our simulations based on parameters for *S. crameri* could be extrapolated to other representatives of the rockfish complex. For instance, most rockfishes used as examples in the previous paragraph have also shown IBD patterns (e.g., Withler et al. 2001; Buonaccorsi et al. 2002, 2004), suggesting that SSMM might have general application. The use of a single estimate of N_e from *S. crameri* to provide both population sizes and mutation rates used during simulations is perhaps the main limitation of our study. Rockfishes are characterized by large life-history variation (Love et al. 2002), making generalizations

difficult. To our knowledge, no other N_e estimates are available for rockfishes, which emphasize the increasing need for demographic genetics studies in conservation.

Notwithstanding the management value of our reference F_{ST}, this was determined from selectively neutral markers, and thus provides limited or no insight into adaptive evolutionary processes of rockfish populations. In general, wide latitudinal ranges characterize the geographic distribution of rockfishes; many of them are found between the coasts of Alaska and California (Love et al. 2002), which may offer ample opportunities of local adaptation given contrasting oceanographic conditions. This fact should be further acknowledged for large-scale spatial management practices. Our simulations with *S. crameri* suggest, however, that high gene flow between adjacent populations should erase any differences in adaptive genetic variation in the long term, even for widely spaced populations.

Lastly, many biological reference points in fishery biology have been proposed using quantitative approaches together with some arbitrary criteria (Quinn and Deriso 1999). Our reference F_{ST}/PA is no exception. Further interaction between fishery biologists, managers and marine geneticists is warranted to determine applicability of our reference F_{ST}/PA to various conservation goals within the rockfish complex, and establish which species face greater or lesser risks of losing genetic diversity.

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General conclusions

From my investigation regarding spatial and temporal scales of genetic change in overfished rockfishes, the following conclusions could be drawn:

1) *Microsatellite DNA:* Microsatellite markers have greatly expanded our knowledge of rockfish biology. Although they have been utilized mainly for population genetics inference during this investigation, they hold promise in other areas such as parentage analysis and future genomic studies. Indeed, novel research using parent-offspring models using microsatellites are delivering important findings on rockfishes' mating system (J. R. Hyde, pers. comm. 2005) and juvenile dispersal at short spatial scales (L. Hauser, pers. comm. 2005).

2) *Spatial genetic structure:* Population differentiation varies enormously across rockfishes, and the duration of their larval stage is apparently not a good predictor of genetic differentiation—canary and darkblotched rockfish (and many others) both possess pelagic stages of 3-6 months in offshore waters; yet, they differ significantly in their genetic structure. Significant life-history variation in the ill-understood larval and juvenile stages might explain differences among rockfishes inhabiting similar habitats. Isolation-by-distance in darkblotched and other rockfishes seem to indicate that effective dispersal is restricted to nearby populations.

3) *Small sample sizes and overall genetic distance:* According to my results with darkblotched rockfish, it is possible to increase the signal of genetic differentiation by pooling samples comprised by only few individuals. This is better done using a criterion based on an absolute genetic distance rather than usual statistical tests, which can generate artifacts. However, this strategy might only work in those species showing isolation-by-distance patterns.

4) *Temporal genetic changes:* Microsatellite data from canary rockfish archived otoliths point to significant genetic changes over a period of 31 years (1973-2003) of fishery data, in contrast to non-significant spatial differentiation. Genetic drift alone could explain these changes, demonstrating that, despite large census population sizes, the size of the breeding population could be much smaller (see conclusion 5). Nevertheless, we found no significant evidence for a linear loss of genetic diversity owing to fishing and the stock's rapid decline in abundance during the period of study. Historical limitations of our archived dataset and non-linear effects of fishing could be alternative explanations.

5) *Effective population size (N_e) and ratio N_e/N:* Differences of three to four orders of magnitude were found between estimates of effective ($\hat{N}_e$) and census population sizes ($\hat{N}$) in both rockfishes under study. Genetic structure and skewed sex ratios could partially account for this discrepancy in darkblotched and canary rockfish, respectively; however, the major effects is a

large variance in reproductive success among individuals. Low $\hat{N}_e / \hat{N}$ might be pervasive among rockfishes and other marine species characterized by high mortalities and fecundities during early stages. This emphasizes the vulnerability of long-lived stocks and reinforces current recovery plans and conservation initiatives.

6) *Rapid appraisal of conservation risk among rockfishes using genetic distance:* Simulations indicate that a significant probability associated with small genetic distance (F_{ST}) does not ensure successful population assignment (PA) of individuals to their site of origin, feeding the discussion of when genetic differentiation is meaningful from a management perspective. The theoretical relationship between F_{ST} and PA suggests that PA is unrealistic (<50%) for $F_{ST} < 0.01$. This result provides a limit of resolution to distinguish which species are at higher risk ($F_{ST} > 0.01$) or lower risk ($F_{ST} < 0.01$) to lose genetic diversity if local populations are extirpated.

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