

Abstract—Rougheye rockfish (*Sebastes aleutianus*) and shortraker rockfish (*Sebastes borealis*) were collected from the Washington coast, the Gulf of Alaska, the southern Bering Sea, and the eastern Kamchatka coast of Russia (areas encompassing most of their geographic distribution) for population genetic analyses. Using starch gel electrophoresis, we analyzed 1027 rougheye rockfish and 615 shortraker rockfish for variation at 29 protein-coding loci. No genetic heterogeneity was found among shortraker rockfish throughout the sampled regions, although shortraker in the Aleutian Islands region, captured at deeper depths, were found to be significantly smaller in size than the shortraker caught in shallower waters from Southeast Alaska. Genetic analysis of the rougheye rockfish revealed two evolutionary lineages that exist in sympatry with little or no gene flow between them. When analyzed as two distinct species, neither lineage exhibited heterogeneity among regions. *Sebastes aleutianus* seems to inhabit waters throughout the Gulf of Alaska and more southern waters, whereas *S. sp. cf. aleutianus* inhabits waters throughout the Gulf of Alaska, Aleutian Islands, and Asia. The distribution of the two rougheye rockfish lineages may be related to depth where they are sympatric. The paler color morph, *S. aleutianus*, is found more abundantly in shallower waters and the darker color morph, *Sebastes sp. cf. aleutianus*, inhabits deeper waters. *Sebastes sp. cf. aleutianus*, also exhibited a significantly higher prevalence of two parasites, *N. robusta* and *T. trituba*, than did *Sebastes aleutianus*, in the 2001 samples, indicating a possible difference in habitat and (or) resource use between the two lineages.

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Genetic variation of rougheye rockfish (*Sebastes aleutianus*) and shortraker rockfish (*S. borealis*) inferred from allozymes

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Information about the biology and population dynamics of rougheye rockfish (*S. aleutianus*) and shortraker rockfish (*S. borealis*) is limited, and uncertainty exists about current stock abundance and long-term productivity. As adults, these two species are similar in appearance, have the same zoogeography, and share the same habitat. They were classified as a single species, *S. aleutianus* (Jordan and Evermann, 1898), until Barsukov (1970) described *S. borealis*. Tsuyuki and Westrheim (1970) also described *S. borealis* that same year (initially as *S. caenaemeticus*), using biochemical methods. The distribution of rougheye rockfish is reported from Japan to southeastern Kamchatka (excluding the Sea of Okhotsk), to Navarin Canyon in the Bering Sea, throughout the Aleutian Islands, and south to San Diego, California (Tokranov and Davydov, 1997). Shortraker rockfish has a similar distribution; however, this species is much more abundant than rougheye rockfish in Russia—eastern Russian *Sebastes* biomass was composed of more than 90% shortraker and less than 1% rougheye rockfish for most regions, excepting the Com-

mander Islands (Tokranov and Davydov, 1997). Both species have been reported at depths to 875 m (Allen and Smith, 1988), although longline (Sigler and Zenger¹) and trawl surveys (NMFS triennial groundfish survey) indicate they are most abundant on the upper continental slope at 300–400 m depths. Krieger and Ito (1999) found the two species difficult to distinguish visually when viewed from a submersible but believed that the highly sedentary adults of both species share the same habitat, preferring substrates of sand or mud and frequent boulders and steep slopes.

Rougheye and shortraker rockfish are highly prized commercially but are particularly sensitive to overexploitation because of slow growth, late maturation, and long life spans. Half of rougheye rockfish are mature at 20

¹ Sigler, M. F., and H. H. Zenger Jr. 1994. Relative abundance of Gulf of Alaska sablefish and other groundfish based on the domestic longline survey, 1989. U. S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-40, 79 p. Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

years of age (McDermott, 1994). Rougheye rockfish have been estimated to attain ages in excess of 200 years and shortraker rockfish in excess of 150 years (Munk, 2001). These two species are currently managed together as the “shortraker-rougheye” assemblage within waters managed under a North Pacific Fishery Management Council (NPFMC) fishery management plan. Commercial catch levels in NPFMC areas of the Bering Sea, the Aleutian Islands, and the Gulf of Alaska averaged 2400 t each year from 1999 to 2001 (Heifetz et al., 2002; Spencer and Reuter, 2002).

The annual catch quota for rockfish and most groundfish managed by the NPFMC is apportioned among five relatively large geographic areas: the eastern, central, and western Gulf of Alaska, the Aleutian Islands, and the eastern Bering Sea. Previous work in the Gulf of Alaska has indicated geographical segregation of the two rougheye species (Moles et al., 1998; Hawkins et al.²). Based on earlier designations of the International North Pacific Fisheries Commission, area boundaries have little biological basis. If the population structure of a particular species has different geographic boundaries than the boundaries of the designated management areas for the species, there is risk of over-harvest. The objective of this study is to examine the population structure of rougheye and shortraker rockfish by using allozyme variation. This is the first population structure study of these two species that encompasses all the North Pacific management areas and most of their biological ranges.

Methods

Collection

Adult rougheye rockfish were collected with bottom trawls from the Gulf of Alaska in 1993, the eastern Bering Sea in 1994, and from the Washington coast in 1998. They were also collected by longline from waters north of Unalaska Island in the Aleutian Islands in 1996, the central Gulf of Alaska and the northwestern Bering Sea near Russia in 1997, and north of Unalaska Island (Aleutian Islands) and in the eastern and central Gulf of Alaska in 2001. Shortraker rockfish were collected with bottom trawls from the Gulf of Alaska in 1993, the eastern Bering Sea in 1994, and by longline in the northwestern Bering Sea near Russia in 1997. Dates, locations, and sample sizes are reported in Table 1 and Figure 1.

Approximately 2–3 mL of liver, heart, and muscle were taken from each fish, temporarily stored in either a freezer (–20°C) or in liquid nitrogen, shipped to the

Auke Bay Laboratory, Alaska, and stored at –80°C. Eye tissue was taken from the 1993 Southeast Alaska samples but was not collected during subsequent sampling efforts because initial experimentation yielded limited results from this tissue. Samples of heart tissue were sent to the University of Alaska for DNA analysis. Only liver tissue was taken from the Shumagin and Aleutian Islands rougheye rockfish samples in 2001 (regions 9b, 14a, and 16a). The right gill arch and a 4-inch section of the gut were sampled for parasite analysis from the rougheye rockfish 2001 Gulf of Alaska samples. These fish were also photographed, preserved in 10% formalin, and shipped to the Alaska Fisheries Science Center for future morphological studies.

Laboratory analysis

Protein enzymes from each sample were separated by horizontal starch-gel electrophoresis as described by Aebersold et al. (1987). Enzymes were screened by staining eye, heart, liver, and muscle tissue on each of six buffer systems (Table 2) by using general staining procedures (Harris and Hopkinson, 1976; Aebersold et al., 1987), and *Sebastes*-specific procedures (Seeb, 1986). Enzyme screening was designed to detect interspecific allelic mobility differences and to identify intraspecific multilocus enzymes by tissue. Therefore, each tissue type from both rougheye and shortraker rockfish were run together on each gel buffer. Of 47 enzymes screened, 23 enzymes representing 29 loci were resolved for all rougheye rockfish except the Russian collection, for which 25 loci were resolved, and the collections from regions 9b, 14a, and 16a, for which only liver samples were taken and 7 loci were resolved (data available from senior author). Twenty-nine loci were resolved for all shortraker rockfish collections except the Russian collection, for which 24 loci were resolved (data available from senior author). The loci used in subsequent analyses and the level of variation are listed in Table 2. Nomenclature for identified loci were assigned according to the American Fisheries Society guidelines for standardization (Shaklee et al., 1990).

Data analysis

Fish sampled from stations in close proximity were combined to form regional collections (Table 1 and Fig. 1). The software package GENEPOP (vers. 3.4, Montpellier University, Montpellier, France) was used to calculate genotypic frequencies for each region and to test for departure from expected Hardy-Weinberg equilibrium frequencies. Homogeneity of allele frequencies among regional collections was tested with log-likelihood ratio analysis (G-test; Sokal and Rohlf, 1981). F_{is} and F_{st} were calculated with FSTAT (Goudet, 1995).

Heterogeneity among the collections and within some of the collections of rougheye rockfish was such that the fish were easily divided into two distinct “types” according to their genotypes at five loci: *ACP**, *IDDH**, *MPI**, *PGM-2**, and *XO** (Table 3 and data available from

² Hawkins, S. L., J. Heifetz, J. Pohl, and R. Wilmot. 1997. Unpubl. data. Genetic population structure of rougheye rockfish (*Sebastes aleutianus*) inferred from allozyme variation. Alaska Fisheries Science Center, Quarterly Report Feature, July–Aug.–Sept. Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

Table 1

Regional group, location, sample size (*n*) of *S. aleutianus*, *S. sp. cf. aleutianus*, U = unknown type of *S. aleutianus*, *S. borealis*, and latitude, longitude, depth, and date of collections.

Region	Location	<i>Sebastes aleutianus</i> (<i>n</i>)	<i>Sebastes aleutianus</i> sp.cf. (<i>n</i>)	U (<i>n</i>)	<i>Sebastes borealis</i> (<i>n</i>)	Lat. N	Long. W	Depth (<i>m</i>)	Date
1	North Washington State	79	3			47.6	125.2	118–421	1998
2	S.E. Alaska, Dixon entrance	20	21		36	54.5	133.5	152–228	1993
3	S.E. Alaska, S. Baranoff Is.	32	16		10	56.0	135.2	176–260	1993
4	S.E. Alaska, Cross Sound	27	4		15	58.1	136.9	77–249	1993
5	S.E. Alaska, Cape Fairweather	19	1	1	38	58.4	139.3	123–241	1993
5a	S.E. Alaska, Cape Fairweather	2	6	2		58.4	140.4	300–400	2001
6	S.E. Alaska, Yakutat	50	3	4	53	59.3	141.2	102–198	1993
6a	S.E. Alaska, Yakutat	6	10	6		59.2	141.1	300–400	2001
7	S.E. Alaska, Cape Suckling	22	0	1	32	59.8	143.4	81–217	1993
7a	S.E. Alaska, Cape Suckling	5	42	2		59.3	143.1	300–600	2001
8	S. of Prince William Sound	4	43			58.2	148.4	300–600	1997
8a	S. E. of Prince William Sound	2	17			59.1	147.2	300–400	2001
9	Kodiak Island, S.W.	12	86		99	56.3	152.1	270–400	1996
9a	Kodiak Island, S.W.	11	0	8		58.0	152.2	140–150	2001
9b	Shumagin Island, S.W.	5	0	2		55.4	159.4	145	2001
10	South of Amlia Island								
	S. between Atka & Amlia Is	0	76		105	51.8	173.9	303–320	1994
	South of Amlia Island	0	12			51.5	173.3	163–650	1996
11	South Atka Pass								
	South Atka Pass	0	22		34	51.7	175.5	309–407	1994
	South of Atka Island	0	25	1	20	51.5	175.1	185–820	1996
12	South Tanaga Island								
	South of Tanaga Island	0	70		57	51.6	177.6	372–381	1994
	West of Tanaga Island	0	24		12	51.4	178.1	90–705	1996
13	North of Semisopochnoi Island	0	28			52.5	180.0	213	1994
14	North Atka Pass	0	30		37	52.1	175.0	108–940	1996
14a	North of Amlia Island	0	11	2		52.5	173.5	230–350	2001
15	N. of Islands of Four Mountains	0	34		12	53.0	170.1	172–630	1996
16	North Unalaska Island								
	North of Unalaska Island	5	0			53.7	167.0	195	1994
	North of Unalaska Island	6	44			53.7	167.0	121–350	1997
16a	North of Unalaska Island	8	3	1		53.6	167.5	85–303	2001
17	northwest Bering Sea, Russia	0	55			60.5	179.3E	390–384	1997
	northwest Bering Sea, Russia				55	60.3	171.4E	457–533	1997

senior author). We identified these types as *Sebastes aleutianus* and *Sebastes sp. cf. aleutianus* (a species that has putatively not been described but is similar to *S. aleutianus*). The *S. aleutianus* type is characterized by individuals with genomes of predominately *ACP**100; *IDDH**100, and *500; *MPI**129; *PGM-2**100, *83, *91, and *117; and *XO**100. The *Sebastes sp. cf. aleutianus* type is characterized by individuals with genomes of predominately *ACP**46; *IDDH**500 and *750; *MPI**100; *PGM-2**83, *74, and *63; and *XO**109. We used 25 loci to perform multidimensional scaling analysis of individual rougheye genotypes to illustrate separation of the two types.

We chose STRUCTURE, a Bayesian clustering model (Pritchard et al., 2000) to gain greater statistical rigor in identifying individual types and possible hybrids of rougheye rockfish. This model seeks to identify populations in a mixture without the availability of baseline samples from the separate populations. The proportions of each individual's genome belonging to the population identified by the model and the separate population allele frequencies are simultaneously estimated. A 90% probability interval is computed for each individual's inferred genome source proportions. For this analysis, we used 25 loci, 100,000 iterations, and a 10,000 burn-in period. This model assumes that loci are in Hardy-

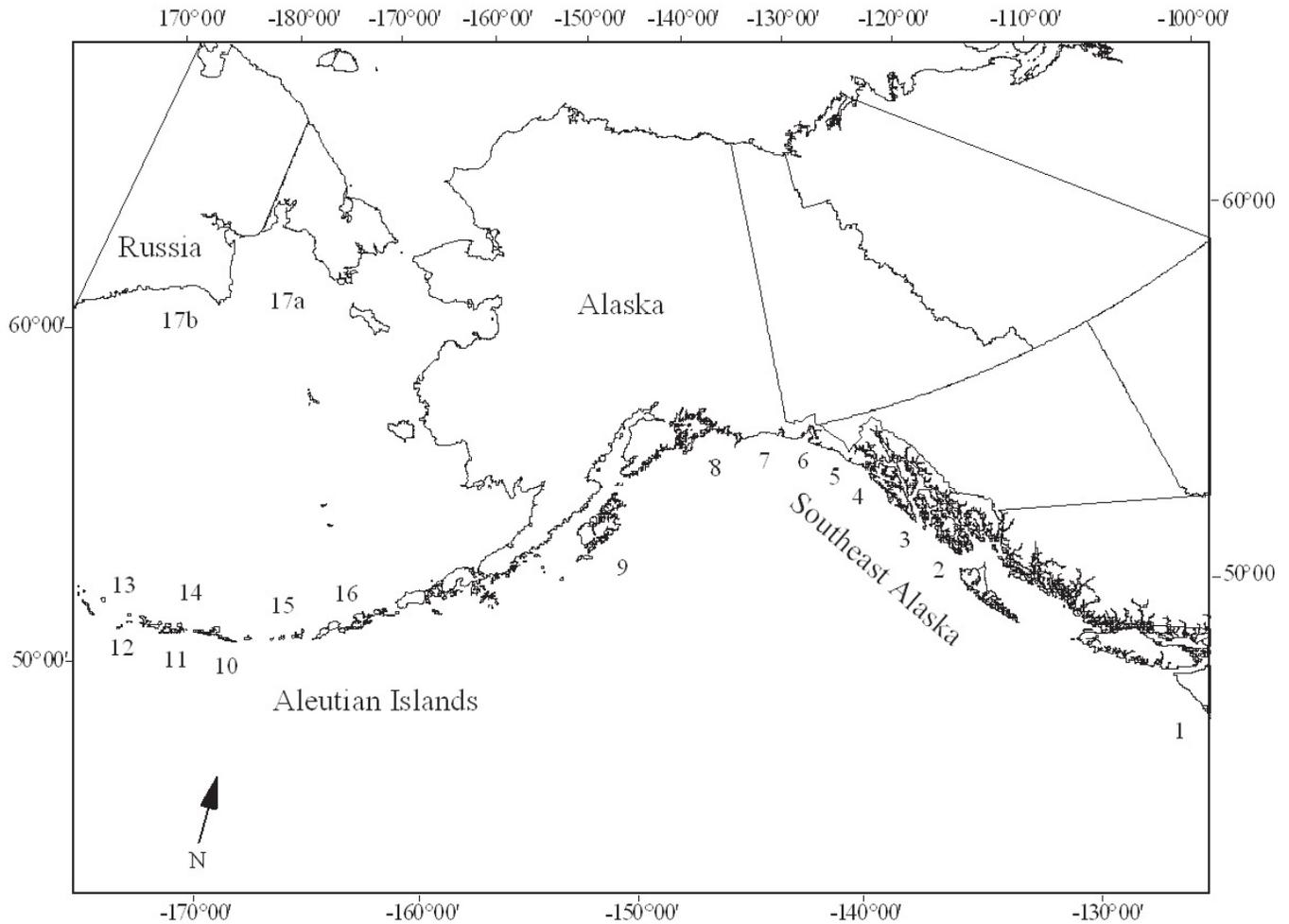


Figure 1

Location of rougheye (*Sebastes aleutianus*) and shorttraker rockfish (*Sebastes borealis*) collection sites, which correspond to locations in Table 1.

Weinberg equilibrium within populations and in linkage equilibrium with one another within populations. These assumptions were tested with the PC program GENEPOP (vers. 3.4, Univ. Montpellier, Montpellier, France).

Regional groups were separated into two groups of rougheye rockfish types according to the multidimensional scaling analysis and Bayesian clustering model and were retested for Hardy-Weinberg equilibrium and homogeneity of allelic frequencies (G-test) among regions for each type. Chord distance (Cavalli-Sforza and Edwards, 1967) for 25 loci was used to assess the overall similarities of allelic frequencies for the two rougheye rockfish types with multidimensional scaling analysis (Rohlf, 2000). Only 25 loci were used because the Russian collection was missing data at 4 loci. Regions 9b, 14a, and 16a were therefore not included in these analyses because of the limited number of loci available.

Because the two rougheye rockfish types exhibited a distinct yet puzzling pattern of distribution—nearly all *S. sp. cf. aleutianus* in the Aleutian Is-

lands, nearly all *S. aleutianus* in the central Gulf of Alaska, and both types in sympatry in Southeast Alaska—we collected rougheye rockfish at different depths in 2001 (regions 5a–9a, 9b, 16a). We ran a Mann-Whitney rank sum test (SigmaStat, vers. 2.0, SPSS, Chicago, IL) to test for significant differences of the mean, standard deviation, and range of depths between the two rougheye rockfish types. A single depth of 350 m was used to approximate depth of catch for the 2001 Southeast Alaska rougheye rockfish collections (regions 5a, 6a, and 7a) because depths were reported only as a range from 300 to 600 m. Had we chosen a deeper average depth in the range, the difference in depth between the two rougheye rockfish types would have been (and in actuality may be) even greater. Because the two rougheye types were found in sympatry, we analyzed the length data to determine if size differences existed between the two types. Linear regressions were used to examine the relationships between length (tip of snout to fork of tail) and depth of capture of both shorttraker rockfish and the two rougheye rockfish types.

Table 2

Enzymes with associated International Union of Biochemistry Numbers (IUBNC), locus name (Shaklee et al., 1990), tissue(s), buffer(s), and level of variability for *Sebastes aleutianus*. RE=both *Sebastes* sp. cf. *aleutianus* and *Sebastes aleutianus*, and SR=*Sebastes borealis*. Tissue: M=muscle; H=heart; and L=liver. Buffers: 1= R (Ridgway et al., 1970); 2 = MF (Markert and Faulhaber, 1965); 3=CA6.1 and 4- CA6.9 (Clayton and Tretiak, 1972, modified pH); 5 = TC (Shaw and Prasad, 1970); and 6 = CAME7.4 (modified from Clayton and Tretiak, 1972). Var. RE and Var. SR: 0 = monomorphic; 1 = frequency common allele >0.95; 2 = frequency common allele <0.95 for at least one region. — = Loci were not reliably scored in that species. + = loci were not reliably scored in all populations and were not used in most analyses.

Enzyme	IUBNC no.	Locus	Tissue	Buffer	Var. RE	Var. SR
Acid phosphatase	3.1.3.2	<i>ACP*</i>	L	3	2	—
Aconitate hydratase	4.2.1.3	<i>mAH*</i>	H	5,6	1	1
		<i>sAH*</i>	L	3,4	2	1
Adenosine deaminase	3.5.4.4	<i>ADA-1*</i>	M,H	3,6	0	2
Adenylate kinase	2.7.4.3	<i>AK*</i>	M,H,L	6	0	0
Alcohol dehydrogenase	1.1.1.1	<i>ADH*</i>	L	3	2 ¹	2
Aspartate aminotransferase	2.6.1.1	<i>sAAT*</i>	L	1	2 ¹	0
		<i>mAAT*</i>	M,H,L	3,4,6	1	1
beta-N-Acetylgalactosaminidase	3.2.1.53	<i>bGALA*</i>	L	4	0 ¹	0
Creatine kinase	2.7.3.2	<i>CK-1*+</i>	H	3,6	1	0
Fumarate hydratase	4.2.1.2	<i>FH*</i>	H,L	5	0 ¹	1
Glucose-6-phosphate isomerase	5.3.1.9	<i>GPI-A*</i>	M,H,L	1,3	1	2
		<i>GPI-B*</i>	M,H	1,3	1	1
Glycerol-3-phosphate dehydrogenase	1.1.1.8	<i>G3PDH*</i>	M	2	0	1
Iditol dehydrogenase	1.1.1.15	<i>IDDH*</i>	L	1	2	—
Isocitrate dehydrogenase	1.1.1.42	<i>IDHP-1*+</i>	H	3	1	1
		<i>IDHP-2*</i>	L	3	1	1
Lactate dehydrogenase	1.1.1.27	<i>LDH*</i>	M,H	3	0	0
Malate dehydrogenase	1.1.1.37	<i>MDH-1*</i>	M,H	3,6	1	—
		<i>MDH-2*</i>	M,H,L	3,4,6	1	1
Malic enzyme	1.1.1.40	<i>mMEP*</i>	M,H	3,6	2 ¹	2
Mannose-6-phosphate isomerase	5.3.1.8	<i>MPI*</i>	H	6	2	2
Dipeptidase (glycyl-leucine)	3.4.-.	<i>PEPA*</i>	M,H,L	2	2	1
Tripeptide aminopeptidase (leu-gly-gly)	3.4.-.	<i>PEPB*</i>	M,H,L	1	0	0
		<i>PEPD*+</i>	M,H	2	—	2
		<i>PEP-LT*+</i>	M,H	2	—	1
Phosphoglucomutase	5.4.2.2	<i>PGM-1*</i>	M,H,L	1,5	2 ¹	2
		<i>PGM-2*</i>	H	5	2	2
6-Phosphogluconate dehydrogenase	1.1.1.44	<i>PGDH*</i>	M,H,L	3	2 ¹	0
Triose-phosphate isomerase	5.3.1.1	<i>TPI-1*</i>	M,H	1,3	0	0
		<i>TPI-2*</i>	M,H	1,3	—	2
Xanthine Oxidase		<i>XO*</i>	L	2	2 ¹	0

¹ *Sebastes* sp. cf. *aleutianus* level of variability was 1.

Parasite analysis

Although not an objective of the study, parasites were opportunistically sampled from the 2001 Gulf of Alaska collections of rougheye rockfish to determine if depth or species subtype might have been a factor in the geographical segregation noted by Moles et al. in 1998. This would also allow us to determine if the parasite data supported results of the current allozyme work. The rougheye rock-

fish were examined for the proportion of fish with the gill parasites *Neobrachiella robusta*, *Trochopus trituba*, or the visceral parasite *Corynosoma* sp. by using the procedures of Moles et al. (1998). A categorical analysis of variance (SAS procedure, CATMOD: vers. 8.02. Cary, NC 1989) was used to test whether parasite prevalence differed among the two types of rougheye rockfish.

Table 3Allelic frequencies of five loci for all samples by type that best distinguish *Sebastes aleutianus* and *Sebastes* sp. cf. *aleutianus*.

Locus	<i>n</i>	Allele					
<i>ACP</i> *		100	46	83			
<i>aleutianus</i>	242	0.896	0.087	0.017			
sp. cf. <i>aleutianus</i>	486	0.094	0.905	0.001			
<i>IDDH</i> *		100	500	750	999		
<i>aleutianus</i>	287	0.73	0.268	0.002	0		
sp. cf. <i>aleutianus</i>	658	0.03	0.507	0.462	0.001		
<i>MPI</i> *		100	129	110			
<i>aleutianus</i>	283	0.343	0.656	0.001			
sp. cf. <i>aleutianus</i>	540	0.74	0.26	0			
<i>PGM-2</i> *		100	83	74	63/69/59**	80	91/117**
<i>aleutianus</i>	270	0.775	0.185	0.005	0	0.002	0.028
sp. cf. <i>aleutianus</i>	586	0.003	0.333	0.508	0.147	0.009	0
<i>XO</i> *		100	109				
<i>aleutianus</i>	295	0.844	0.156				
sp. cf. <i>aleutianus</i>	660	0.011	0.989				

** indicates pooled alleles.

Results

Shorthead rockfish and roughey rockfish had different common alleles (fixed) for 10 of 29 loci examined (*sAH**, *CK-A1**, *GPI-A**, *G3PDH**, *IDHP-2**, *PEPA**, *PEPB**, *PEP-LT**, *PGM-2**, and *SOD**). These are inexpensive markers that can be used to differentiate shorthead rockfish from roughey rockfish when precise field identification, particularly in younger fish, is necessary but difficult.

Shorthead rockfish

Nine loci (31%) were monomorphic for all regions, 11 loci (38%) were variable (with the frequency of the common allele greater than 0.95 for all regional groups), and 9 loci (31%) had a common allele frequency of less than 0.95 for at least one regional group. For the Russian collection, data were unavailable from five loci (*FH**, *mIDHP**, *MPI**, *PGM-2**, and *TPI-2**). Average heterozygosity of each regional group fell within a narrow range of 0.09–0.11, and produced an overall average for 29 loci of 0.10. All regional genotypic proportions closely agreed with those expected under Hardy-Weinberg equilibrium; of 128 chi-square tests, only four (3%) differed significantly ($P < 0.05$) from expected values. No significant ($P < 0.05$) heterogeneity was detected with G-tests among

regional groups, and thus no subpopulations or stock structure was evident with this suite of allozymes.

Although no genetic differentiation was detected among shorthead rockfish throughout their geographic distribution, size of fish and depth of capture differed between shorthead rockfish from the Aleutian Islands and those from Southeast Alaska. Aleutian Island shorthead rockfish were significantly smaller (mean 43.6 cm [\pm SD 7.0], range: 24–70 cm) and were caught in deeper water (309–407 m) than Southeast Alaska shorthead rockfish (mean 66.5 cm [\pm SD 10.5], range: 45–101 cm at 138–260 m depths). A regression of fish length on depth of capture yielded a significant r^2 value of 0.452 ($P < 0.001$).

Roughey rockfish

Significant departure from Hardy-Weinberg equilibrium occurred in 37 out of 226 possible tests (16%); a value greater than the 11 that would be expected by chance alone at the $P = 0.05$ level of probability (Table 4). Thirty-six of the departures were due to an absence of heterozygotes, a situation known as the Wahlund effect, which typically indicates the presence of a mixture of populations for presumably neutral genetic loci. Most of the departure from Hardy-Weinberg expectations occurred at *ACP**, *IDDH**, *MPI**, *PGM-2**, and *XO**. Only

Table 4
Loci not in Hardy Weinberg equilibrium ($P < 0.05$). N/A = insufficient sample size for analysis.

Location	Mixture	<i>S. aleutianus</i> ¹	<i>S. sp. cf. aleutianus</i> ¹
Dixon Entrance	ACP, sAH, IDDH PGM-2, XO	ACP, IDDH	ACP, IDDH
S. Baranof Island	ACP, IDDH, XO PGM-1, PGM-2	ACP, PGM-1	None
Cross Sound	ACP, IDDH, XO	None	N/A
Cape Fairweather	ACP, PGM-2	ACP	N/A
Yakutat	ACP, MPI PGM-2, XO	MPI	N/A
Cape Suckling	None	None	N/A
Prince William Sound	IDDH, PGM-2, XO	N/A	None
Kodiak	ACP, IDDH PGM-2, XO	None	ACP
Amlia Island	None	N/A	None
South Atka Pass	MPI	N/A	MPI
South Tanaga Island	PEPA, PGM-2	N/A	PEPA
North Semisopchnoi Island	IDDH	N/A	IDDH
North Atka Pass	None	N/A	None
N. Is. Of Four Mountains	None	N/A	None
North Unalaska Island	ACP, PGM-2, XO	ACP, XO	None
Washington	sAH, IDDH, MPI PGM-2	sAH, MPI	N/A
Russia	None	N/A	None

¹ As determined from the program STRUCTURE (Pritchard et al., 2000).

PGM-2* in the South Tanaga Island sample was due to an excess of heterozygotes.

Inbreeding coefficients (F_{is}) indicated deviation from panmixia. The values ranged from -0.050 for *IDHP-1** to 0.772 for *ACP**. The mean value over all loci in all collections was 0.140 . Statistically significant F_{is} values were found at *ACP** (0.521), *MPI** (0.135), *PGM-2** (0.109), and *XO** (0.524). All were the result of heterozygote deficiencies. The mean F_{is} value for the *S. aleutianus* type collections dropped to 0.062 and for the *S. sp. cf. aleutianus* types, to 0.048 .

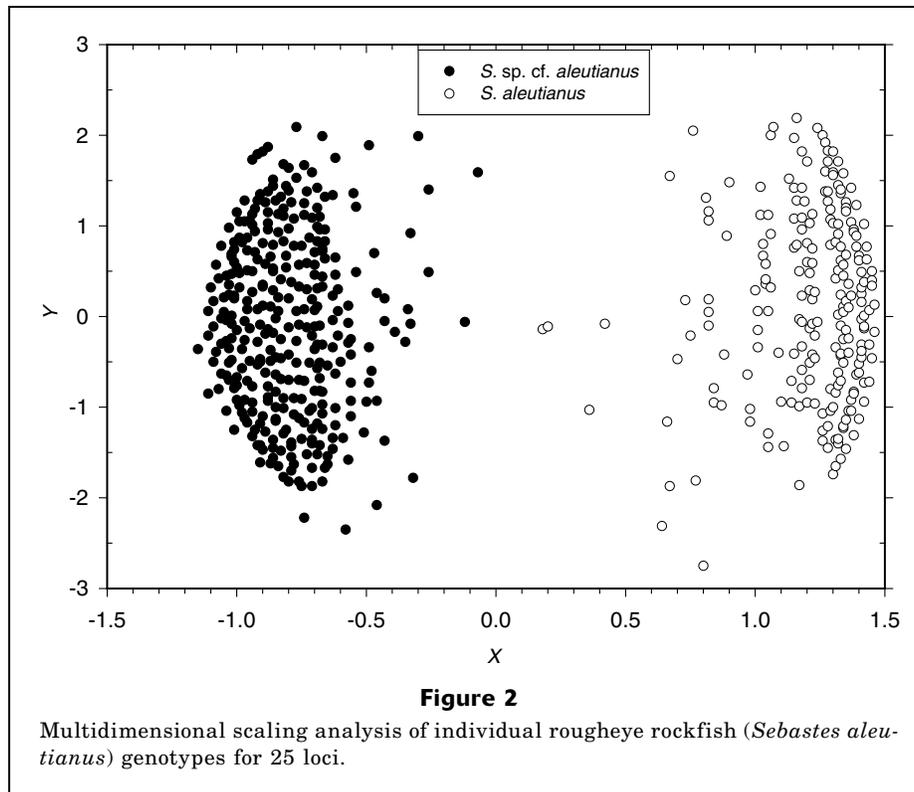
Eight of the loci showed statistically significant F_{st} values: *sAAT** (0.007), *ACP** (0.572), *sAH** (0.037), *IDDH** (0.189), *MDH-2** (0.007), *MPI** (0.123), *PGM-2** (0.206), and *XO** (0.551). The mean F_{st} value for all loci in all collections was 0.215 . When analyzed by pure *S. aleutianus*-type and *S. sp. cf. aleutianus*-type, the mean F_{st} values dropped to 0.013 and 0.012 , respectively.

Two rougheye types

The results of the rougheye rockfish analyses allowed us to segregate rougheye rockfish individuals into two types: *S. aleutianus* and *Sebastes sp. cf. aleutianus*. Multidimensional scaling analysis with individual genotypes (Fig. 2) yielded two distinct clusters with little

overlap. This outcome was confirmed by the Bayesian clustering model in STRUCTURE (Pritchard et al., 2000), which identified two types. We calculated the inferred source proportions of genomes for 1027 individuals using 25 loci that were scored in most individuals. One hundred sixty-six individuals were missing data for more than 30% of the 25 loci used and were omitted from the analysis. Most of the individuals had a very high proportion of their genome from one type; for 851 individuals, the program assigned at least 0.80 of the individual's genes to one of the two ancestral lines, and all had an upper 90% probability limit that included 1.0. These fish were likely all purebreds. Ten individuals had an inferred proportion of ancestry from one lineage of less than 0.80 and two did not include an upper probability interval of 1.0. These individuals were possibly hybrids. If any of these 10 individuals were actual hybrids of the two rougheye rockfish types, none were of the first generation (i.e., heterozygotes at all differentiating loci).

Significant differences of allele frequencies (G-test) were detected between the two types at 14 loci: $P < 0.001$ for *sAAT**, *ACP**, *ADH**, *sAH**, *IDDH**, *MDH-2**, *mMEP**, *MPI**, *PGDH**, *PGM-1**, *PGM-2**, and *XO**; and $P < 0.05$ for *mAAT** and *GPI-B**. When the two types were analyzed independently by area (Table 4), all but two collections were in Hardy-Weinberg equilibrium



(South Tanaga Island *Sebastes* sp. cf. *aleutianus* type, $P=0.042$, and North Unalaska Island *S. aleutianus* type, $P=0.023$). The G-test analysis indicated no heterogeneity among regions except for the Russian sample, which was significantly different from all other samples ($P<0.05$). Average heterozygosity was 0.09 for *S. aleutianus* and 0.08 for *Sebastes* sp. cf. *aleutianus*.

A significant difference in overall depth of capture ($P<0.001$) was detected between *Sebastes* sp. cf. *aleutianus* (mean 330+ m) and *S. aleutianus* (mean 208 m). We obtained both shallow and deep collections from the central Gulf of Alaska. The fish captured at shallow depths, 77–249 m (regions 4–7, 9a, 9b, $n=134$), were nearly all (94%) *S. aleutianus*, whereas the deeper dwelling fish, 270–600 m (regions 5a–7a, 8, 8a, 9, $n=204$), were mostly (87%) *Sebastes* sp. cf. *aleutianus*. Both types were captured, some within a single haul, in southern Southeast Alaska (regions 2 and 3, $n=89$) at depths of 150–260 m (Fig. 3).

A highly significant correlation of fish length (15–65 cm) and depth of capture (77–260 m) was detected for *S. aleutianus* in Southeast Alaska, with smaller fish in shallower water and larger fish in deeper water ($r^2=0.415$, $P<0.001$). No length-depth trend was noted for *Sebastes* sp. cf. *aleutianus*.

Results of the parasite analysis for the 2001 rougheye rockfish showed that *Sebastes* sp. cf. *aleutianus* had a significantly higher prevalence of both *Neobrachiella robusta* ($P=0.003$) and *Trochopus trituba* ($P=0.022$) than did *S. aleutianus* (Table 5).

Discussion

The most notable conclusion of our study was that two genetically distinct types of rougheye rockfish exist. This conclusion corroborates prior biochemical studies in which Tsuyuki et al. (1968) and Tsuyuki and Westrheim (1970) conducted hemoglobin electropherogram analyses on *S. aleutianus* and *S. caenaematicus* (= *S. borealis*) and detected four blood types. Three blood types characterized *S. aleutianus*—two distinct types and a rare hybrid type. The fourth type characterized *S. borealis*. Seeb (1986) examined allozymes from several species of North Pacific rockfish and found two color morphs of rougheye rockfish fixed for alternate alleles at three loci. At one of the loci, *ACP**, we detected a small percentage of a shared allele, likely because of our larger sample size. We were unable to resolve the other two loci, *GAP** (IUBNC no. 1.2.1.12 Glyceraldehyde-3-phosphate dehydrogenase,) and *GAM** (B-Galactosaminidase). Although we are unable to report fixed loci differences between the two rougheye rockfish types, we did detect significant allele frequency differences at nearly half of the loci examined. Allelic mobilities of *Sebastes aleutianus* were similar to those of Seeb's "*Sebastes aleutianus*," and allelic mobilities of *Sebastes* sp. cf. *aleutianus* were similar to Seeb's "*Sebastes aleutianus* unknown." Because simultaneous hemoglobin and allozyme studies have never been done, we are currently unable to correlate allozyme types with the blood types reported by Tsuyuki et al. (1968) and Tsuyuki and Westrheim (1970).

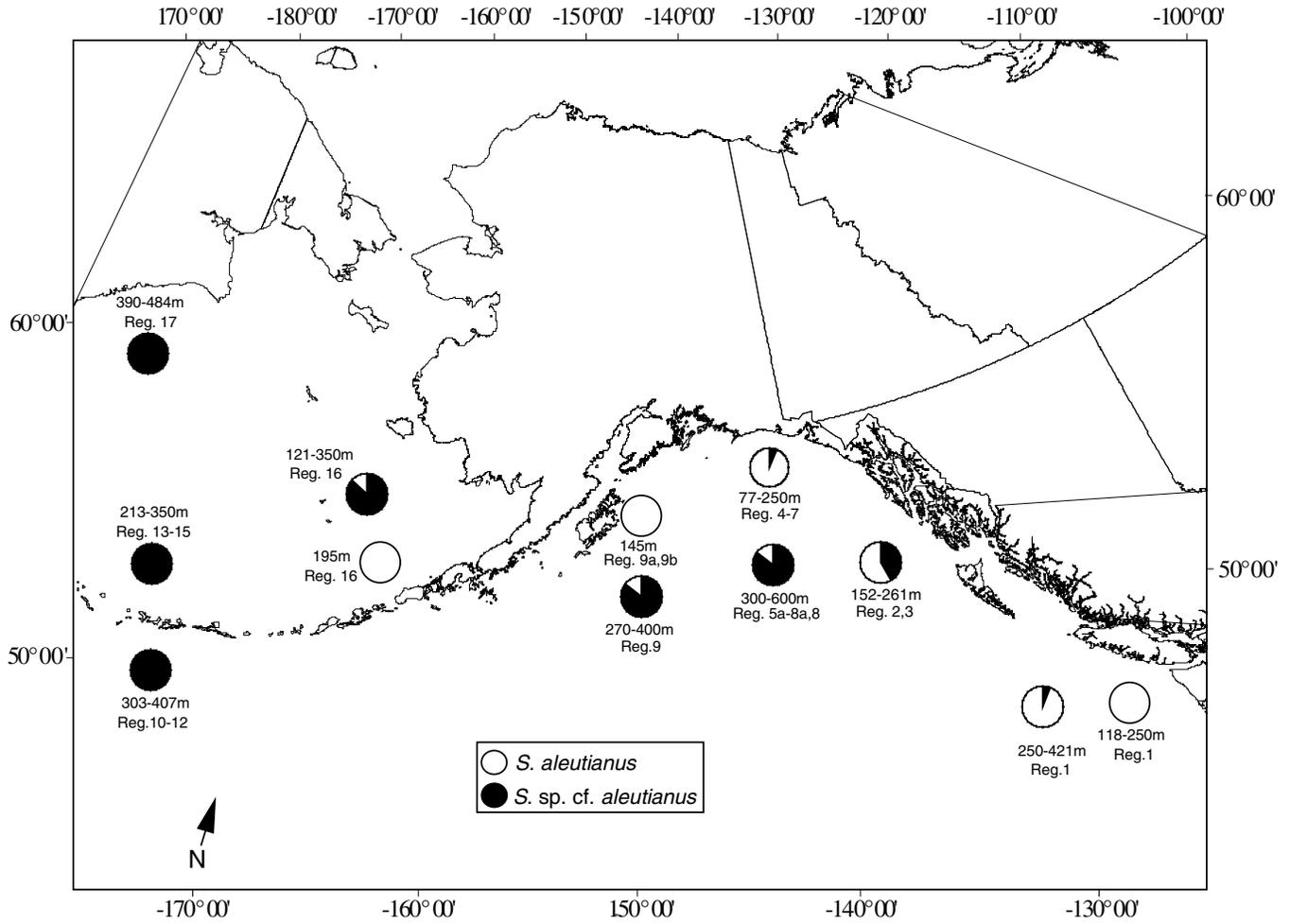


Figure 3
Proportions of *Sebastes aleutianus* and *S. sp. cf. aleutianus* in relation to depth of capture.

Table 5
Prevalence of parasites (percentage) in both rougheye rockfish types and results of categorical analysis of variance.

	Parasite prevalence		Significance probability	
	<i>sp. cf. aleutianus</i> (n=61)	<i>S. aleutianus</i> (n=18)	Type	Size of fish
<i>Neobrachiella robusta</i>	0.57	0.11	0.003*	0.442
<i>Trochopus trituba</i>	0.49	0.17	0.022*	0.339
<i>Corynosoma sp.</i>	0.90	0.83	0.448	0.326

The Cavalli-Sforza-Edwards (CSE) chord distance (for 29 loci) between the two *S. aleutianus* types, 0.35 (SD=0.05), was a value comparable to that for other closely related rockfish species. Seeb (1986) reported CSE distances between rockfish species ranging from 0.07 to 0.75 for 28 loci. Identical mobilities at the majority of loci indicated a close relationship between the two types, which probably existed as a single type at an earlier geologic time. Given that Tsuyuki and

Westrheim (1970) detected (2%) hybrids of the two blood types and we did not detect fixed differences between the two rougheye rockfish types, some gene flow may be occurring. However, the low effective number of migrants and the sympatric distribution of the population indicate that the gene flow is limited. Because rockfish have internal fertilization, sibling species may co-occur and there is little chance of cross-fertilization of gametes.

The initial objective of our study did not include collection of morphological data, but in light of the genetic differences detected, and the color morphs detected by Seeb (1986), morphology of the two lineages should be more closely examined. Upon processing the 2001 fish, we noted that many were easily identified as either light or dark in color, although some appeared intermediate. The obvious light-colored individuals were all found genetically to be *S. aleutianus*, whereas the darker specimens were typically *Sebastes* sp. cf. *aleutianus*. Although Tsuyuki and Westrheim (1970) reported no distinguishing meristic or morphometric characters between *S. aleutianus* blood types, Seeb (1986) separated rougheye rockfish morphologically and by color into two groups: one that was light pink and had spines under the orbit of the eye (*S. aleutianus*); the other was darker and had a considerable area of black on the mouth and jaw and often lacked orbital spines (*S. aleutianus* unknown). The lack of orbital spines in *Sebastes* sp. cf. *aleutianus* is an important observation because this feature is a key characteristic in distinguishing *S. aleutianus* from *S. borealis*.

Initial observations of the distribution of the 1993 rougheye samples displayed a pattern of predominately *S. aleutianus* in the Gulf of Alaska and almost entirely *S. sp. cf. aleutianus* in the Aleutian Islands. A parasite study (Moles et al., 1998) performed on the same rougheye rockfish reported a significantly greater ($P < 0.05$) prevalence of three parasites in the Aleutian Island samples. Upon close examination of the depth of the sample collection, we noted that the Aleutian Islands samples were collected in deeper waters than those collected in the Gulf of Alaska. Thus subsequent sampling strategies focused on a possible depth niche. Parasite data from both the shallow and deep water 2001 rougheye collections showed a significant prevalence of two parasites in the deeper host, *Sebastes* sp. cf. *aleutianus* (Table 5). The prevalence of the parasite *T. tributata* may be dependent on host habitat. Because the hosts (two *aleutianus* types) exhibited significantly different prevalences of the parasite *T. tributata*, they may be using different resources (different diets) and (or) exhibiting ecological segregation (Moles et al., 1998).

Both *Sebastes aleutianus* types are found in the Gulf of Alaska and occur in sympatry, although the majority of *S. sp. cf. aleutianus* are distributed at deeper depths (Fig. 3). Bathymetric segregation has been noted in other closely related rockfish. *Sebastes fasciatus* and *Sebastes mentella* in the Atlantic Ocean elude abundance estimates because of a lack of easily identifiable morphological characteristics. In the laboratory, these two species are nearly fixed at alternate alleles at the allozyme locus MDH*. A bathymetric segregation is detected, where *S. fasciatus* is found at depths of 132–315 m, *S. mentella* at depths greater than 355 m, and both species and possible hybrids at intermediate depths (Rubeck et al., 1991). Another species pair, *Sebastes carnatus* and *Sebastes chrysomelas*, are both shallow-dwelling species (depth less than 20 m) that coexist only in a narrow zone of overlap that separates exclusive depth

ranges. Factors organizing their segregation are largely behavioral because both species expand their depths in the absence of the other species (Larson, 1980). Rough-eye rockfish may exhibit a similar strategy. Deep sampling efforts near Washington state have indicated that the distribution of *S. sp. cf. aleutianus* may diminish in southern ranges and it appears that the distribution of *S. aleutianus* does not extend to the western Aleutian Islands and Asia. This pattern of distribution has been noted in other closely related species, such as the northern and southern species of *Lepidopsetta* (Orr and Matarese, 2000). The poorly understood ontogenetic and seasonal movements of rougheye and other rockfish further confound the picture. For example, *S. altivelis* and *S. alascanus* were long thought to be deep-dwelling and shallow-dwelling congeneric species, respectively. It is now known that *S. altivelis* is a permanent deepwater resident, whereas *S. alascanus* settles in shallow water, then migrates to deep water with the onset of sexual maturity. Competition between these two species may be reduced by size differences; for example, where they are sympatric, *S. alascanus* is much larger than *S. altivelis* (Vetter and Lynn, 1997). Although length data for rougheye rockfish in sympatry are limited, a single haul ($n=29$) near Dixon Entrance (depth of capture = 213 m) yielded both types, and nearly all the *S. aleutianus* individuals were much larger (mean 56.3 cm) than *Sebastes* sp. cf. *aleutianus* (mean 37.2 cm). No age data are currently available to add insight into these results. The trend of smaller fish in shallower waters and larger fish in deeper waters was significant for *S. aleutianus* in the Gulf of Alaska. This was not detected for *Sebastes* sp. cf. *aleutianus*, although their full depth range may not have been sampled. Perhaps only younger *Sebastes* sp. cf. *aleutianus* were collected, and the older, larger fish are deeper and remain unsampled. It would be beneficial to analyze juvenile rougheye rockfish to ascertain genetic type composition at different depths.

A significant size difference existed between fish in the Gulf of Alaska and those of the Aleutian Islands, especially among shortraker rockfish. Aleutian fish were smaller, despite collection at greater depth. Growth and age of maturation differences among differing latitudes and longitudes have been noted in other rockfish species (Westrheim, 1973; Archibald et al., 1981; Field, 1984; Lunsford, 1999). The size difference among shortraker rockfish has been previously noted (Orlov, 2001; Matala, 2004) and raises more questions than it answers. It is still unknown whether these differences are caused by different age classes or regional ecological differences.

Allozyme data did not reveal heterogeneity within either rougheye rockfish type or within shortraker rockfish throughout the sampled geographic range. Although no heterogeneity was detected with our suite of allozyme loci, other genetic markers, such as microsatellite loci, may provide finer resolution of population structure. A recent study of shortraker microsatellite variation revealed geographically restricted homogeneity among allele frequencies—a model consistent with the assump-

tion of limited movement (Matala, 2004). Conversely, Orlov (2001) proposed a synopsis of horizontal adult migration (with increased size of shortraker rockfish at spawning grounds) and oceanic dispersal of larvae and juveniles.

In conclusion, it appears there are species-level differences between the two rougheyed rockfish types. We have considered the darker morph *Sebastes* sp. cf. *aleutianus* as the new type. The paler *S. aleutianus* morph conforms more to the original *S. aleutianus* type description, an individual of which was captured at a 55-m depth in the Gulf of Alaska. It is likely that the distribution of the new species *S. sp cf aleutianus* stretches from the Gulf of Alaska and west to Asia. The distribution of *S. aleutianus* encompasses the Gulf of Alaska and extends south to California, and the species is found in more shallow waters where it is sympatric with *S. sp cf aleutianus*. An understanding of the basic life history, distribution, and biomass of a species is critical for successful resource management. Ito (1999) suggested that the major fisheries management survey effort is the NMFS Gulf of Alaska triennial trawl survey, which may be inadequate to assess the shortraker-rougheyed rockfish assemblage because its multispecies sampling design covers mostly depths less than 200 m. This survey may, therefore, be completely missing *Sebastes* sp. cf. *aleutianus* altogether. An important consideration for management is knowledge of exploitation rates. Given the sensitivity of long-lived rockfish species to over-exploitation, basic biological studies should be undertaken of these species to understand characteristics such as growth, maturity, and natural mortality.

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