Stock Separation of Five Rockfish Species Using Naturally Occurring Biochemical Genetic Markers

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ABSTRACT–Stock relationships of five commercially important rockfish (genus Sebastes*) species were studied using biochemical genetic information developed through electrophoresis. Samples of Pacific ocean perch,* S. alutus, *were collected off Washington and Oregon and in the Gulf of Alaska from Dixon Entrance to Kodiak. Three stock groups were recognized for Pacific ocean perch. One exists off the Washington and Oregon coasts, while another is found in the Gulf of Alaska. A third previously unrecognized stock was tentatively identified off Prince William Sound. Samples of canary rockfish,* S. pinniger; *yellowtail rockfish,* S. flavidus; *chilipepper,* S. goodei; *and bocaccio,* S. paucispinis, *were collected off the California, Oregon, and Washington coasts. Two stocks may exist for canary rockfish–one located off northern California and southern Oregon and the other located off northern Oregon and Washington. Only one stock group per species was recognized for yellowtail rockfish, chilipepper, and bocaccio. The relationship of the different species to each other and the general applications of the techniques of electrophoresis to work in marine fisheries are also discussed.*

Introduction

Biochemical genetics has gained increasingly widespread acceptance as a stock separation tool during the last decade. The analytical techniques of biochemical genetic (i.e., electrophoretic) studies are based on the distributions among individuals and populations of simple genetic protein variants. These variants, which have been shown to be coded for by single genes, are inherited according to simple genetic principles, remain unchanged throughout the life of the organism, and are passed on from generation to generation (Utter et al., 1974). A significant difference between biochemical genetic techniques and the traditional types of stock separation techniques is that the biochemical techniques detect only genetic differences. Genetic and environmental influences may not be distinguishable by traditional methods such as meristic studies, size distribution, or year-class strength comparisons. However, the two approaches, biochemical and traditional, can be valuable complements to each other.

Electrophoretic data allow a population or a species to be characterized by its frequency of biochemical genetic variants. Groups of individuals which share a common gene pool will have similar gene frequencies for these variants. However, if isolation between groups occurs, these frequencies may shift either by natural selection or such random processes as genetic drift, and the isolates may develop significantly different gene frequencies. These gene frequency differences can be used as an effective stock separation tool to identify noninterbreeding groups.

This report presents the findings of the biochemical genetic data collected in conjunction with the 1977 rockfish survey. The primary focus concerned *Sebastes alutus,* Pacific ocean perch, but significant amounts of data were also collected from four of the other rockfish species (*S. pinniger,* canary rockfish; *S. flavidus,* yellowtail rockfish; *S. paucispinis,* boccacio; and *S. goodei,* chilipepper). Stock relationships of *S. alutus* have been studied extensively (Westrheim, 1970, 1973, 1975; Fadeev, 1968; Lisovenko, 1964; Lyubimova, 1965), but very little is known of the stock relationships of the other rockfish. This paper compares the genetic data with the previously published stock relationships of *S. alutus* and outlines possible stock relationships based solely on genetic data for the other four species. The genetic relationships among the five species are also investigated. In addition, the role of genetic studies in the management of rockfish and other marine fisheries is discussed.

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Methods and Materials

Samples for electrophoresis were collected on board ship concurrently with the collection of other rockfish data and samples. Approximately 10 g of muscle and liver tissue were extracted from each fish, placed in a 4- × 4-inch Ziploc[1] bag, identified by specimen number, and frozen as soon as possible. Sex and length data were taken on each fish; in some cases weight data and otoliths were also taken. Each fish could then be identified by genotype, sex and length (and by weight and age when available). All fish from an individual haul were combined and identified by a vessel and haul number (Tables 1-5).

Sampling was designed to include 50 fish from three different depth zones within each stratum of the major distributional areas of a particular species. However, in practice the genetic samples were collected whenever an adequate number of fish were caught in a particular haul. As a result the sampling localities are heavily concentrated in the areas of maximum abundance. Not all depth zones nor strata are represented for each species. The size of the confidence intervals around the gene frequency estimates is a direct function of the number of fish examined according to the following formula:

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1.—Allele frequencies for Pacific ocean perch, *Sebastes alutus*, collected during 1977.

Stratum	Vessel and haul number	Depth (fm)	Location		Mo. and day	Sample size	AGP	PGM	PGI-2				6PG	ADH					IDH-2			
			N. Lat.	W. Long.			100	100	100	92	87	79	100	100	66	46	110	164	100	80	68	120
8	23-094	95	43°50′	124°51′	8-23	49	.52	52	1.00	—	—	—	.99	.99	—	—	—	.01	1.00	—	—	—
8	23-090	140	43°09′	124°45′	8-21	49	.68	.43	1.00	—	—	—	.97	.96	.03	—	.01	—	.99	—	—	.01
9	23-097	112	44°08′	124°57′	8-24	50	.58	.41	1.00	—	—	—	.97	.97	.03	.01	—	—	—	—	—	—
9	22-167	117	44°53′	124°28′	9-01	48	.60	.42	1.00	—	—	—	.98	.95	.03	.01	.01	—	.99	—	—	.01
10	22-179	112	45°14′	124°18′	9-06	50	.58	.38	.96	.04	—	—	.96	.97	.03	—	—	—	1.00	—	—	—
10	22-172	114	45°04′	124°20′	9-05	50	.55	.49	1.00	—	—	—	.98	.97	.03	—	—	—	.99	—	.01	—
10	04-203	120	45°00′	124°22′	9-01	20	.47	.55	1.00	—	—	—	.95	.97	.03	—	—	—	1.00	—	—	—
10	23-105	127	45°13′	124°21	8-27	49	.68	.49	1.00	—	—	—	.97	.99	.01	—	—	—	1.00	—	—	—
10	23-107	165	45°25′	124°27′	8-28	49	.58	.51	1.00	—	—	—	.98	.98	—	—	.02	—	1.00	—	—	—
10	23-104	190	45°07′	124°33′	8-27	46	.61	.43	1.00	—	—	—	.97	.97	.03	—	—	—	.99	—	.01	—
10	04-209	203	45°11′	124°35′	9-05	15	.47	.55	1.00	—	—	—	.95	.97	.03	—	—	—	1.00	—	—	—
10	04-210	214	45°10′	124°31′	9-05	22	.80	.36	1.00	—	—	—	.98	1.00	—	—	—	—	1.00	—	—	—
10	04-208	222	45°02′	124°41′	9-05	13	.61	.46	1.00	—	—	—	.88	.96	.04	—	—	—	.96	—	.04	—
11	04-222	102	46°18′	124°37′	9-08	50	.59	.47	1.00	—	—	—	.96	.99	.01	—	—	—	1.00	—	—	—
11	22-186	105	46°26′	124°32′	9-08	50	.60	.40	.99	—	—	.01	1.00	.96	.02	.01	.01	—	.99	.01	—	—
11	22-191	106	46°36′	124°37′	9-09	48	.64	.40	1.00	—	—	—	.98	.97	.02	—	.01	—	1.00	—	—	—
11	20-014	118	45°53′	124°41′	9-01	47	.67	.46	1.00	—	—	—	1.00	.96	.04	—	—	—	1.00	—	—	—
11	22-192	150	46°36′	124°37′	9-09	43	.59	.53	1.00	—	—	—	.97	.89	.07	.02	—	.02	1.00	—	—	—
11	22-187	158	46°26′	124°33′	9-08	50	.70	.54	1.00	—	—	—	.98	.98	.01	—	.01	—	1.00	—	—	—
11	20-013	209	45°54′	124°45′	9-01	14	.61	.40	1.00	—	—	—	.93	.89	.07	—	.04	—	.92	—	.04	.04
11	23-113	211	45°54′	124°46′	8-29	20	.60	.50	1.00	—	—	—	.90	1.00	—	—	—	—	1.00	—	—	—
12	04-234	172	46°49′	124°54′	9-14	49	.56	.42	1.00	—	—	—	.99	.99	.01	—	—	—	1.00	—	—	—
12	04-241	222	47°13′	124°56′	9-15	47	.69	.51	1.00	—	—	—	.94	.97	.03	—	—	—	1.00	—	—	—
12	22-203	225	47°10′	124°59′	9-15	20	.47	.55	1.00	—	—	—	.95	.97	.03	—	—	—	1.00	—	—	—
13	04-251	70	48°21	124°57′	9-18	41	.59	44	1.00	—	—	—	.99	.97	.02	—	—	.01	1.00	—	—	—
13	23-132	96	48°13′	124°58′	9-04	32	.64	.47	1.00	—	—	—	.98	.97	.03	—	—	—	1.00	—	—	—
13	23-128	106	47°50′	125°08′	9-21	50	.49	.44	1.00	—	—	—	.95	.99	.01	—	—	—	1.00	—	—	—
13	23-134	115	48°19′	125°52′	9-04	49	.54	.41	1.00	—	—	—	.99	.98	.02	—	—	—	1.00	—	—	—
13	22-209	118	47°11′	124°35′	9-05	14	.68	.60	1.00	—	—	—	.90	.83	17	—	—	—	1.00	—	—	—
13	23-131	121	47°59′	125°37′	9-03	50	.49	.48	1.00	—	—	—	.98	1.00	—	—	—	—	1.00	—	—	—
13	22-220	168	47°53′	125°14′	9-22	50	.57	.40	1.00	—	—	—	.99	.97	.03	—	—	—	1.00	—	—	—
13	04-267	172	48°09′	125°42′	9-22	50	.54	.43	1.00	—	—	—	.99	.96	.02	—	—	.01	.99	—	.01	—
13	23-125	180	47°35′	125°05′	9-02	51	.66	.35	1.00	—	—	—	.90	.94	.04	—	—	.01	.99	—	.01	—
13	04-268	229	48°10′	125°43′	9-22	50	.65	.40	1.00	—	—	—	.93	.95	.05	—	—	—	.98	—	—	.02
17	23-011	87	56°60′	136°06′	7-08	48	.65	.50	1.00	—	—	—	1.00	.95	.04	—	—	—	.95	—	—	.04
17	23-016	104	57°59′	137°51′	7-10	46	.59	.48	1.00	—	—	—	1.00	.98	.02	—	—	—	1.00	—	—	—
19	23-026	175	59°10′	141°15′	7-14	24	.56	.55	1.00	—	—	—	.98	.98	.02	—	—	—	.98	—	.02	—
19	23-032	156	59°37′	143°05′	7-16	48	.62	.55	1.00	—	—	—	1.00	.94	.06	—	—	—	.97	.01	.01	—
20	23-056	109	59°14′	147°39′	7-25	49	.46	.48	.99	—	.01	—	1.00	.85	15	—	—	—	.99	—	.01	—
20	23-055	103	59°05′	147°54′	7-27	18	.42	.39	.97	—	.03	—	1.00	.97	.03	—	—	—	.97	—	.03	—
20	23-1000	—	—	—	7-00	50	.38	.54	.98	—	.02	—	1.00	.89	.09	.02	—	—	.99	—	.01	—
21	23-049	70	59°53′	149°07′	7-25	49	.50	.61	1.00	—	—	—	1.00	.96	.04	—	—	—	.99	—	.01	—

95% Confidence interval $= \pm 2$ SE

$$= 2\sqrt{\frac{p\,(1\text{-}p)}{2N}}$$

where N is the number of fish, p is the frequency of the allele in question, and $(1\text{-}p)$ is the combined frequency of all other alleles at that locus. An attempt was made to collect 50 fish from each haul to obtain reasonable confidence intervals around the estimates, but in many cases this was not possible since fewer than 50 fish were caught.

Sampling was most intensive for Pacific ocean perch. A total of 1,385 fish were taken off the Oregon and Washington coasts. An additional 400 fish were collected in the Gulf of Alaska aboard the Polish research vessel *Profesor Siedlecki* (Stratum 14-21, Table 6). Fewer individuals of the other four species were captured. Samples were taken from a total of 620 canary rockfish, 593 yellowtail rockfish, 506 chilipepper, and 327 bocaccio. (See Tables 1-5 for sampling localities.)

Samples were shipped frozen to the Northwest and Alaska Fisheries Center of the National Marine Fisheries Service (NMFS) in Seattle, Wash., for analysis. Electrophoresis was conducted following the methods of Utter et al. (1974). During electrophoresis, the various proteins were subjected to an electric current and migrated different distances through a starch gel according to their relative charge and structure. After electrophoresis, histochemical staining techniques described in Allendorf et al. (1977) were used to visualize the proteins.

For each electrophoretically detectable locus the mobility (distance travelled) of the most common protein type found in Pacific ocean perch was used as a standard. The mobility of all other variants or alleles for any of the rockfish

Table 2.—Allele frequencies for canary rockfish, *Sebastes pinniger*, collected during 1977.

Stratum	Vessel and haul number	Depth (fm)	Location N. Lat.	Location W. Long.	Mo. and day	Sample size	PGM 100	PGI-2 100	PGI-2 87	PGI-2 79	PGI-2 69	ME-1 100	ME-1 65	ME-1 96	TO 100	TO 25	TO 66	SDH 100	SDH 50	IDH-1 100	ME-2 100	ME-2 110	ME-2 93
6	25-065	64	40°27′	124°35′	8-13	50	.93	—	.98	—	.02	.93	.07	—	1.00	—	—	1.00	—				
6	23-081	78	40°58′	124°22′	8-18	28	.86	.02	.93	—	.05	.91	.09	—	1.00	—	—	.98	.02	1.00	1.00	—	—
7	23-088	47	42°50′	124°40′	8-21	14	.89	—	1.00	—	—	1.00	—	—	1.00	—	—	1.00	—	1.00	1.00	—	—
7	23-089	78	42°59′	124°48′	8-21	50	.88	.02	.96	—	.02	.94	.06	—	.99	—	.01	1.00	—	1.00	1.00	—	—
8	23-091	115	43°25′	124°40′	8-22	50	.87	—	.97	—	.03	.88	11	.01	1.00	—	—	1.00	—	1.00			
9	23-100	82	44°35′	124°33′	8-25	50	.92	—	.99	—	.01	.93	.07	—	.99	—	.01	1.00	—	1.00	.98	—	.02
9	04-192	92	44°24′	124°46′	8-29	18	.85	—	.94	.03	.03	1.00	—	—	1.00	—	—	1.00	—	1.00	1.00	—	—
10	20-009	101	45°50′	124°38′	9-01	42	.92	—	.99	—	.01	.87	13	—	1.00	—	—	.99	.01	1.00	1.00	—	—
11	23-116	46	46°12′	124°17′	8-30	50	.85	.03	.94	.01	.02	.91	.08	.01	1.00	—	—	1.00	—	1.00	.98	.02	—
11	04-225	65	46°21′	124°26′	9-08	50	.88	.02	.98	—	—	.91	.09	—	1.00	—	—	1.00	—	1.00			
11	23-118	69	46°34′	124°29′	8-30	28	.87	—	.95	—	.05	.91	.09	—	1.00	—	—	1.00	—	.98	.98	.02	—
11	22-191	106	46°36′	124°37′	9-09	35	.89	—	.99	.01	—	.94	.06	—	1.00	—	—	—	—	.99	1.00	—	—
12	23-120	44	46°46′	124°26′	8-31	50	.78	—	1.00	—	—	.94	.06	—	.99	.01	—	1.00	—	1.00	.99	—	.01
12	04-243	110	47°29′	124°53′	9-16	33	.95	.02	.95	.01	.02	.88	12	—	1.00	—	—	1.00	—	.98	1.00	—	—
13	23-129	85	47°59′	125°12′	9-03	22	.95	—	1.00	—	—	.88	.12	—	1.00	—	—	1.00	—	1.00	1.00	—	—
13	22-231	88	48°01	125°28′	9-25	50	.86	—	.98	—	.02	.93	.07	—	1.00	—	—	1.00	—	.99	1.00	—	—

Table 3.—Allele frequencies for yellowtail rockfish, *Sebastes flavidus*, collected during 1977.

Stratum	Vessel and haul number	Depth (fm)	Location N. Lat.	Location W. Long.	Mo. and day	Sample size	PGI-2 87	PGI-2 105	PGI-2 69	6PG 100	6PG 95	6PG 107	6PG 125	6PG 110	MDH-1 100	PEP-2 105	IDH-1 100	IDH-1 80	IDH-1 89	IDH-2 100	IDH-2 110	IDH-2 120	IDH-2 86
7	22-135	55	42°52′	124°41′	8-23	39	1.00	—	—	—	.64	.33	.03	—	.99	.88	1.00	—	—	.99	—	—	.01
7	23-089	78	42°59′	124°48′	8-21	50	1.00	—	—	—	.62	.37	—	.01	.99	.86	1.00	—	—	1.00	—	—	—
7	25-017	80	42°34′	124°42′	8-05	50	1.00	—	—	—	.53	.47	—	—	.99	.86	.99	.01	—	.94	—	—	.06
9	22-169	69	44°50′	124°18′	9-02	50	1.00	—	—	—	.46	.53	—	.01	.99	.90	1.00	—	—	.98	—	.01	.01
9	22-159	80	44°34′	124°34′	8-31	50	.99	.01	—	—	.58	.42	—	—	.98	.90	1.00	—	—	1.00	—	—	—
11	23-116	46	46°12′	124°17′	8-30	37	.99	.01	—	—	.55	.44	.01	—	.98	.85	.99	.01	—	1.00	—	—	—
11	22-190	54	46°37′	124°26′	9-08	38	1.00	—	—	—	.49	.51	—	—	1.00	.91	.99	—	.01	1.00	—	—	—
11	04-222	102	46°18′	124°37′	9-08	50	1.00	—	—	.01	.63	.35	.01	—	1.00	.92	1.00	—	—	.98	—	—	.02
11	23-118	69	46°34′	124°29′	8-30	50	1.00	—	—	—	.55	.44	—	.01	1.00	.82	1.00	—	—	.99	.01	—	—
12	04-244	86	47°30′	124°52′	9-16	50	1.00	—	—	—	.61	.39	—	—	.98	.90	1.00	—	—	.96	—	—	.04
12	04-239	129	47°13′	124°54′	9-15	48	.98	—	.02	—	.65	.35	—	—	1.00	.91	1.00	—	—	1.00	—	—	—
13	04-258	61	48°16′	124°54′	9-21	31	1.00	—	—	.02	.46	.50	.02	—	1.00	—	1.00	—	—	.98	—	—	.02
13	04-260	66	48°15′	125°06′	9-21	50	1.00	—	—	—	.60	.40	—	—	.99	.90	1.00	—	—	.99	—	—	.01

Table 4.—Allele frequencies for bocaccio, *Sebastes paucispinis*, collected during 1977.

Stratum	Vessel and haul number	Depth (fm)	Location N. Lat.	Location W. Long.	Mo. and day	Sample size	PGM 100	PGI-1 100	PGI-2 87	ADH 46	IDH-1 100
1	04-011	100	34°13′	119°43′	7-06	32	1.00	0.58	1.00	0.55	1.00
2	22-011	62	34°49′	120°48′	7-15	50	0.99	0.51	0.98	0.63	0.97
3	22-045	95	36°25′	121°60′	7-21	50	0.99	0.60	0.98	0.58	1.00
4	04-130	69	38°12′	123°18′	8-03	12	1.00	0.59	1.00	0.54	1.00
5	22-110	80	39°08′	123°56′	8-08	28	0.98	0.62	1.00	0.53	1.00
5	22-125	94	39°42′	123°59′	8-11	21	1.00	0.69	1.00	0.68	1.00
5	23-073	96	39°13′	123°57′	8-16	27	1.00	0.50	1.00	0.59	0.98
5	23-078	98	40°04′	124°15′	8-17	57	0.99	0.54	0.98	0.51	1.00
7	23-089	78	42°59′	124°48′	8-21	50	0.97	0.55	1.00	0.57	1.00

species was calculated relative to this common allelic form of Pacific ocean perch which was designated with a mobility of 100. An allelic protein that migrated half as far as the common protein would be designated 50. In the case of multiple forms of the same enzyme, a hyphenated number is attached to the protein abbreviation. A list of the 21 routinely scored loci for the rockfish population survey is given in Table 7 (along with the abbreviation) and the tissue in which the locus was best resolved.

Average genetic heterozygosity ($\bar{H}$) or the average proportion of the genome heterozygous per individual (Selander and Johnson, 1973) was estimated for each of the five species by the formula

$$\bar{H} = (L - \sum_{i=1}^{L} \sum_{j=1}^{A_i} p_{ij}^2) / L$$

where L is the number of loci examined, A_i is the number of alleles at a particular locus, and P_{ij} is the frequency of the jth allele at the ith locus. This is a good measure of the amount of total genetic variation found within a population or a species.

To quantify the relationships between species, similarity coefficients (S) (Rogers, 1972) were calculated between all pairs of species. The coefficient is defined as

$$S = 1 - \frac{1}{L} \sum_{i=1}^{L} \left[\tfrac{1}{2} \sum_{j=1}^{A_i} (p_{ijx} - p_{ijy})^2 \right]^{1/2}$$

where L is the number of loci, A_i is the number of alleles at the ith locus, and p_{ijx} and p_{ijy} are the frequencies of the jth allele at the ith locus in species x and y, respectively. The index ranges from one (for identical allele frequencies) to zero (for those which have no alleles shared in common). These values were used to construct a dendrogram using standard numerical taxomonic techniques (Sneath and Sokal, 1973).

Results and Discussion

Genetic Data For Each Species

This report mainly concerns geographic variation of gene frequencies, although other patterns of variation were also considered. Clines of gene frequencies with depth were investigated to test if gene frequencies could be significantly correlated with depth. No such clines were found for any species at any locus. Age data from otolith readings were available for certain Pacific ocean perch samples from fish taken by the *Profesor Siedlecki*. These data were used to examine the possibility that genotype frequencies varied significantly with age. Again no such relationships were found. Further analyses showed no significant differences between sexes where those data were available.

The collected gene frequencies for each haul examined were tested against the Hardy-Weinberg equilibrium. Significant deviations from the expected values could indicate random sampling errors, an incorrect genetic model for a particular locus, some type of selection, or a mixture of noninterbreeding populations. No consistent significant deviations were detected at any locus for any species.

Pacific Ocean Perch

Pacific ocean perch was the most variable of all the species studied, with a heterozygosity value equal to 0.060. This value is approximately equal to the

Table 5.—Allele frequencies for chilipepper, *Sebastes goodei*, collected during 1977.

Stratum	Vessel and haul number	Depth (fm)	Location N. Lat.	Location W. Long.	Mo. and day	Sample size	6PG 107	6PG 110	6PG 104	PGM 100	PGI-2 87	PGI-2 100	PGI-2 105
1	04-018	56	34°07′	119°57′	7-08	15	.97	—	.03	1.00	.98	.01	.01
2	22-005	58	34°40′	120°48′	7-14	50	.97	.01	.02	1.00	1.00	—	—
2	22-006	108	34°40′	120°51′	7-14	50	.98	.01	.01	.98	1.00	—	—
2	04-046	120	34°27′	120°39′	7-13	24	.98	—	.02	1.00	1.00	—	—
3	04-098	56	36°51′	122°06′	7-22	44	.98	—	.01	1.00	.98	—	.01
3	22-050	100	36°52′	122°12′	7-22	50	.98	—	.02	1.00	1.00	—	—
3	04-096	178	36°48′	122°08′	7-22	16	1.00	—	—	1.00	1.00	—	—
4	04-113	62	37°59′	123°19′	7-31	37	.97	—	.03	.98	1.00	—	—
4	04-133	110	38°24′	123°33′	8-04	25	.96	—	.04	1.00	1.00	—	—
4	22-100	120	38°46′	123°50′	8-06	34	.98	—	.02	1.00	1.00	—	—
4	04-123	124	38°04′	123°31′	8-01	49	.95	—	.05	1.00	1.00	—	—
4	04-124	175	38°04′	123°32′	8-02	12	1.00	—	—	1.00	1.00	—	—
5	22-113	89	39°29′	123°58′	8-09	50	.98	.02	—	.99	.98	.01	.01
5	23-078	98	40°04′	124°15′	8-17	50	.98	—	.01	1.00	1.00	—	—

Table 6.—Boundaries for the geographic areas.

Geographic stratum	Latitudinal (N) boundaries	Geographic stratum	Latitudinal (N) boundaries	Geographic stratum	Latitudinal (N) boundaries
1	34°09′-34°30′	9	44°08′-45°00′	17	56°00′-58°00′
2	34°30′-36°00′	10	45°00′-45°50′		
3	36°16′-37°07′	11	45°50′-46°44′		Longitudinal (W) boundaries
4	37°07′-38°49′	12	46°44′-47°30′		
5	38°49′-40°16′	13	47°30′-48°26′	18	136°00′-140°00′
6	40°25′-41°47′	14	48°26′-52°00′	19	140°00′-144°00′
7	41°47′-43°00′	15	52°00′-54°00′	20	144°00′-148°00′
8	43°00′-44°08′	16	54°00′-56°00′	21	148°00′-152°00′

Table 7.—A list of the enzymes, their abbreviations, and the tissues routinely screened for variation in all five of the *Sebastes* species. Since different genes may code for the same product, each is given a numerical indentifier which follows the abbreviation; i.e., IDH-2 is the second locus coding for isocitrate dehydrogenase.

Enzyme	Abbreviation	Best tissue
Alcohol dehydrogenase	ADH	Liver
Aspartate aminotransferase	AAT	Liver
Creatine kinase	CK	Muscle
Beta-glucuronidase	BGD	Liver
Alpha-glycerophosphate dehydrogenase	AGP	Muscle
Isocitrate dehydrogenase	IDH-1	Muscle
	IDH-2	Liver
Lactate dehydrogenase	LDH	Muscle
Malate dehydrogenase	MDH-1	Muscle
	MDH-2	Muscle
Malic enzyme	ME-1	Muscle
	ME-2	Muscle
Peptidase	PEP-1	Muscle
	PEP-2	Muscle
6-phosphogluconate dehydrogenase	6PG	Muscle
Phosphoglucomutase	PGM	Muscle
Phosphoglucose isomerase	PGI-1	Muscle
	PGI-2	Muscle
Phosphomannose isomerase	PMI	Muscle
Sorbitol dehydrogenase	SDH	Liver
Tetrazolium oxidase	TO	Liver

average for all vertebrate species (Selander and Johnson, 1973). Previously only two biochemical genetic variants had been reported in the literature. These were the AGP (alpha-glycerophosphate dehydrogenase) and PGM (phosphoglucose mutase) loci (Johnson et al., 1972). In this study genetic polymorphisms were found at 15 loci. Four of the 15 polymorphic loci showed variant frequencies that were useful in population characterization: 6PG (6-phosphoglucose dehydrogenase), ADH (alcohol dehydrogenase), PGI-2 (phosphoglucose isomerase-2), and IDH-2 (isocitrate dehydrogenase-2). Allelic frequencies of these four loci and those of AGP and PGM (both highly variable and potentially valuable for stock identification) are given in Table 1.

The most obvious differences in gene frequencies occur among large geographic groups of samples. The data suggest the existence of at least three major stock groups. 6PG separates a Gulf stock from the Washington-Oregon (WA-OR) stock group, while ADH, PGI-2, and AGP separate a subgroup in the Gulf of Alaska from the other Gulf samples and from the Washington-Oregon samples.

Variants of 6PG occur consistently at low frequencies along the Washington-Oregon coast, but the variant is absent in all Gulf samples except one (23-16). Chi-square heterogeneity tests were performed to test for significant intra-group variation. No significant differences occurred so that frequencies can be pooled yielding:

Stock group	N	Frequency	95% Confidence interval
WA-OR	1,385	0.96	0.95-0.97
Gulf	332	1.00	0.99-1.00

The 95 percent confidence intervals between the two groups do not overlap. A chi-square contingency test between the groups was highly significant (χ^2_{1df} =13.75, P <0.01). These significant differences indicate that there is probably no long-term gene flow between the two stock groups.

Differences in the frequencies of several markers indicate that there is a subdivision of Pacific ocean perch stocks within the Gulf of Alaska. Allele frequencies in hauls 23-56, 23-55, and 23-1,000 suggest the existence of an isolated population located off Prince William Sound in the central Gulf of Alaska, since ADH (66) variants appear at a high frequency in the Prince William Sound area as follows:

Stock group	N	Frequency	95% Confidence interval
Other Gulf	215	0.040	0.021-0.059
Prince William Sound	117	0.103	0.063-0.143

The 95 percent confidence intervals do not overlap when the frequencies are calculated to three significant figures. The differences are further substantiated by a highly significant chi-square contingency test between the two groups (χ^2_{1df} =10.39, P <0.01). Other evidence for the uniqueness of the Prince William Sound hauls exists in the AGP and PGI-2 frequencies. AGP frequencies for the Prince William Sound hauls and other Gulf hauls are given below (heterogeneity was tested before pooling):

Stock group	N	Frequency	95% Confidence interval
Other Gulf	215	0.59	0.54-0.64
Prince William Sound	117	0.42	0.36-0.48

The confidence intervals do not overlap and the chi-square contingency test is highly significant (χ^2_{1df} =10.79, P <0.01). Although not significant by itself, the PGI-2 frequencies provide another piece of evidence for the separation of the Gulf stocks, since the PGI-2 (87) allele occurs only in the three Prince William Sound hauls. The geographic relationships of the stock group separations based on the preceding evidence is shown on Figure 1.

Westrheim (1970, 1973) initially concluded that two stocks of Pacific

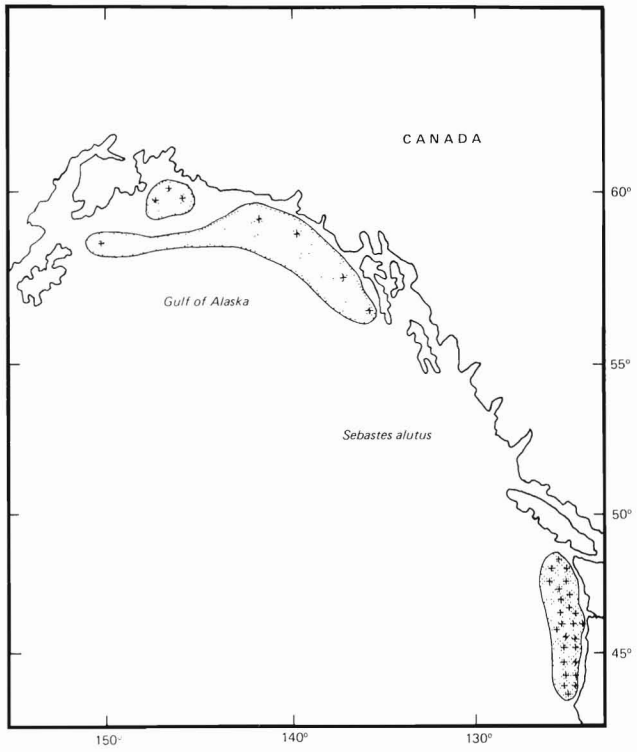

Figure 1.—Sampling localities and stock relationships for Pacific ocean perch based on biochemical genetic data.

ocean perch existed in the northeast Pacific, and tentatively categorized these stocks into British Columbia and Gulf types with the dividing line near Dixon entrance (lat. 54°30'N). These conclusions were based on differential size distributions, year-class strengths, and age-length relationships; he suggested that southeast Gulf fish were more similar to west Gulf fish than to British Columbia fish. However, a different relationship was indicated by more recent data on length-maturity relationships (Westrheim, 1975); i.e., southeast Gulf fish were more similar to British Columbia fish than to west Gulf fish.

The data presented here support the general contention of at least two major stock groups. The two groups identified genetically by 6PG variant frequencies (Washington-Oregon versus Gulf) are presumably analogous to Westrheim's British Columbia and Gulf stocks, although no British Columbia samples were analyzed in this study. These data also strongly indicate the existence of an additional stock group located off Prince William Sound in the central Gulf area that has not previously been identified.

Johnson et al. (1972) observed a nonrandom distribution of PGM phenotypes with depth, where a significantly higher number of heterozygotes (than expected under the Hardy-Weinberg equilibrium) occurred with increasing depths. The data presented here showed no such trend. Chi-square statistics were used to test the observed values against the Hardy-Weinberg expected values, and only one sample out of the 42 collected had a significant deviation from the expected values. No particular PGM phenotype was favored with increasing depth. Preliminary electrophoretic data collected by Henry Tsuyuki (Canadian Department of Environment, Fisheries and Marine Services, Vancouver, B.C., pers. commun. December 1977) also suggested a cline in AGP frequencies with depth off Vancouver Island. When AGP frequencies gathered in this study were plotted against depth, there was no significant correlation ($r^2 = 0.06$). Thus, neither PGM nor APG showed any frequency trends with increasing depth.

The cumulative data support the hypothesis that Pacific ocean perch in the Gulf of Alaska form a series of local populations which only partially intermix (Fadeev, 1968). Fadeev also contended that seasonal migrations are perpendicular to the shelf rather than parallel to it. As the Alaska sampling was only cursory, samples should be gathered and analyzed from all areas of the Gulf of Alaska, including the western Gulf region, the Dixon Entrance area, and the British Columbia coast. More samples are needed in the Prince William Sound region to accurately delineate the boundaries between stocks.

Canary Rockfish

This report provides the first published record of protein polymorphisms for canary rockfish (Table 2). A. G. Johnson (NMFS, Port Aransas, Tex.) recorded a PGM polymorphism in an unpublished report[2]; this polymorphism was substantiated in this study. PGI-2 appeared polymorphic with a total of four alleles at that locus. ME-1 was also moderately polymorphic. Other low frequency variants occurred at the TO, SDH, IDH-1, and ME-2 loci. Overall, canary rockfish had relatively few variants and no high frequency variants. The heterozygosity value (0.022) is low relative to other vertebrates.

The genetic data of different hauls were quite similar and no clear stock separations were apparent such as those observed in Pacific ocean perch. There were, however, two suggestions of situations with possible reduced gene flow. The hauls from strata 6-8 (northern California, lat. 40°25'-44°08'N) completely lacked the variant PGI-2(79). This variant occured in low frequency in strata 9, 11, and 12 (lat. 44°08'-45°00' and 45°50'-47°30'N). Only one sample was taken from stratum 10 (lat. 45°00'-45°50'N), and no variants were observed. No variants were observed in the two hauls from stratum 13 (lat. 47°30'-48°26'N). If strata 6-8 are compared with 9-13 by a contingency test, a chi-square value of 3.2 is generated which is significant at the $P = 0.10$ level.

Some isolation may also exist between the deep and shallow samples based on apparent differences of frequencies of PGM (100). An example occurs in stratum 12. Haul 23-120 has a frequency of 0.78 (95% confidence interval = 0.68-0.85) and was collected at a depth of 44 fathoms. Haul 4-243 has a frequency of 0.95 (0.98-0.87) and was from a depth of 110 fathoms. These confidence intervals do not overlap, suggesting a significant reduction in gene flow.

These two trends are suggestive of some structuring of populations (Fig. 2), but both are inconclusive without further sampling. More samples need to be collected from the deep and shallow extremes within strata, and from specific regions between strata.

Yellowtail Rockfish

No polymorphisms have been previously reported in the literature for yellowtail rockfish. One highly polymorphic system (6PG with five alleles) and one moderately polymorphic system (peptidase) were observed. Low frequency variants were found at PGI-2, MDH-1, IDH-1, and IDH-2 (Table 3). The average heterozygosity for yellowtail rockfish was 0.040.

Data are consistently similar among strata. The one possible exception is haul 4-239 which was the only collection in the deeper ranges (129 fathoms) and possessed a unique allele expressed at a low frequency at the PGI-2 locus. Yellowtail rockfish must be regarded as one homogeneous group within the sampling area without further sampling; this relationship is graphically illustrated in Figure 3.

Bocaccio

Bocaccio had two highly polymorphic loci (PGI-1 and ADH) and an average heterozygosity of 0.056. The data for these and three low frequency variants are given in Table 4.

[2]A. G. Johnson. 1975. Additional data on the biochemical classification of fishes of the family Scorpaenidae. Unpubl. manuscr., 11 p. Panama City Laboratory, NMFS, NOAA, 3500 Delwood Beach Road, Panama City, FL 32401.

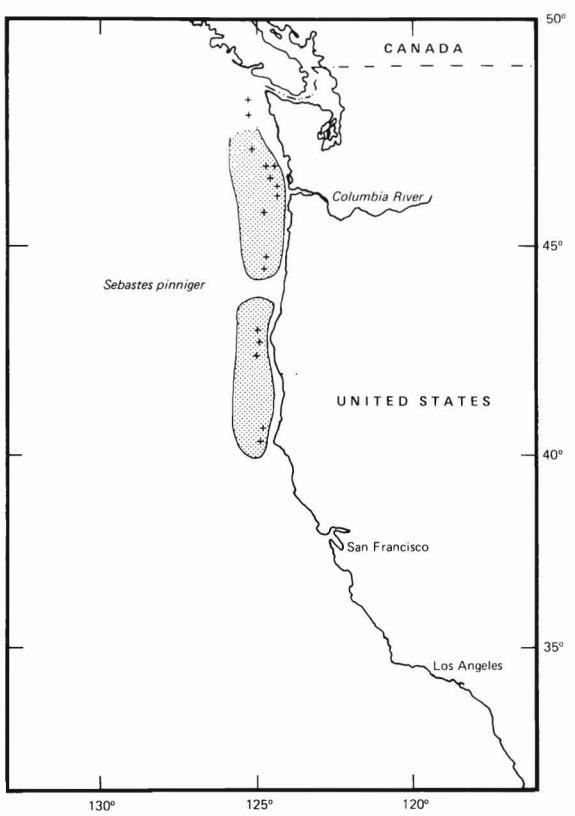

Figure 2.—Sampling localities and possible stock relationships for canary rockfish based on biochemical genetic data.

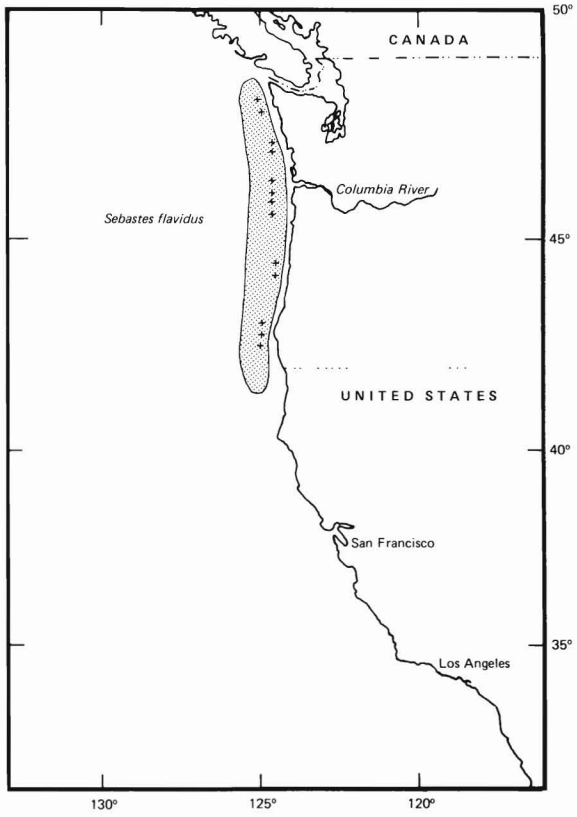

Figure 3.—Sampling localities and stock relationships for yellowtail rockfish based on biochemical genetic data.

The data strongly suggest no genetic differentiation between samples. The samples range over a wide geographic area (strata 1-7, lat. 34°09′-43°00′N), but the alleles have consistently overlapping confidence intervals. The data are especially convincing since the species is so highly polymorphic at two loci. One would not expect these polymorphic loci to remain statistically identical without some gene flow between areas. The sampling areas (and stock relationships) are shown in Figure 4.

Chilipepper

This species is unique for the very low levels of variation associated with it, as reflected in the 0.004 heterozygosity value. This level of variation is unusual, especially in a species with large populations and no barricades to migration. Low frequency variants appeared consistently only at 6PG with an average frequency of 0.02 (Table 5). Rare variants occurred at PGM and PGI-2. All other systems were monomorphic. Sampling localities are shown in Figure 5.

Given the low amount of variation, it is not surprising that no population subdivision was found. Other stock separation techniques may show local differentiation and prove more effective than electrophoresis for this species.

Species Relationships

The five species of rockfish can be compared genetically in terms of their relative genetic similarity to each other. Several indices have been developed for genetic similarity and nearly all of them range from 0 to 1. A similarity of 0 is generated when no alleles are shared in common, while a similarity of 1 indicates that all alleles are not only identical but occur at identical frequencies. Typically populations of one species will show similarities ranging from 0.90 to 1.00; species in the same genus will have similarities ranging downward from 0.90. Rogers' (1972) similarity index was used in this study, and a matrix of the values obtained is given below:

	1	2.	3.	4.	5.
1 Pacific ocean perch	1.000				
2. Canary rockfish	0.565	1.000			
3. Yellow-tail rockfish	0.566	0.755	1.000		
4. Bocaccio	0.353	0.695	0.712	1.000	
5. Chili-pepper	0.567	0.928	0.847	0.846	1.000

The similarity values can be visualized in a dendrogram generated from standard numerical taxonomic techniques (Sneath and Sokal, 1973) (Fig. 6).

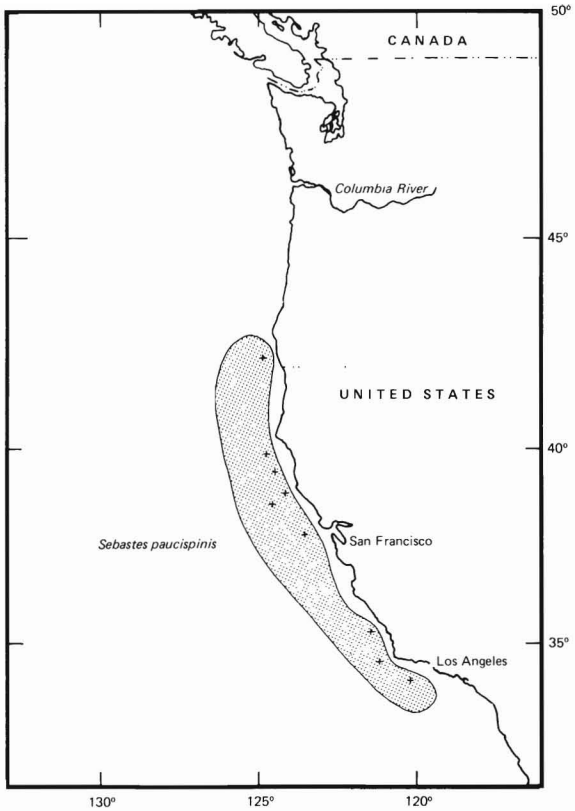

Figure 4.—Sampling localities and stock relationships for bocaccio based on biochemical genetic data.

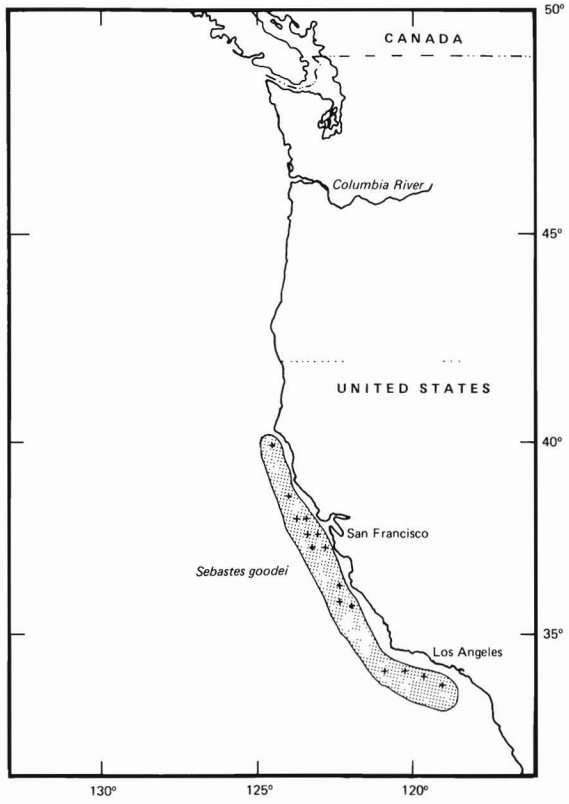

Figure 5.—Sampling localities and stock relationships for chilipepper rockfish based on biochemical genetic data.

As can be seen from the dendrogram and similarity values, canary rockfish and chilipepper are genetically the closest, clustering at 0.93. This is a very close relationship for nonsibling species. Yellowtail rockfish and bocaccio join in at the 0.81 and 0.75 levels, respectively. Pacific ocean perch is the most distinct lineage joining the other species at the 0.51 level.

The amount of variation associated with a particular species may reveal information concerning the relative age and population structuring of that species. The best measure of the amount of variation found within a species is the average genetic heterozygosity ($\bar{H}$), which represents the average proportion of the genome heterozygous per individual. As mentioned previously vertebrates in general range from 0 to 10 percent with an average of about 6 percent heterozygosity. The values for the five rockfish species are:

Species	$(\bar{H})$
Pacific ocean perch	0.060
Canary rockfish	0.022
Yellowtail rockfish	0.040
Bocaccio	0.056
Chilipepper	0.004

Pacific ocean perch and bocaccio have the highest amount of variation, but both fall in the normal range as compared with other vertebrate species. Chilipepper is especially notable for its low heterozygosity value.

Explaining the amount of variation in a species has been and continues to be a major difficulty in the field of population genetics. However, the data from such diverse organisms as man and salmonids support the theory that the variation can best be explained by random processes (Allendorf and Utter, 1979). This theory holds that the amount of variation is dependent on: 1) the effective population size of the species (based on the number of breeding males and females); 2) the age of the lineage since the population was at a low level of abundance; and 3) the dispersal ability of the species (Soule, 1976). It is reasonable to assume that effective population sizes, although very difficult to quantify, are relatively large in most marine species. Small effective population sizes are characteristic of isolated populations such as island dwellers or populations with very skewed sex ratios and low numbers. The age of the lineage from the last severe reduction in numbers provides the most plausible explanation for the differing amounts of variation in rockfish. By this theory the species with the largest population sizes and the oldest lineage will have the highest amount of variation.

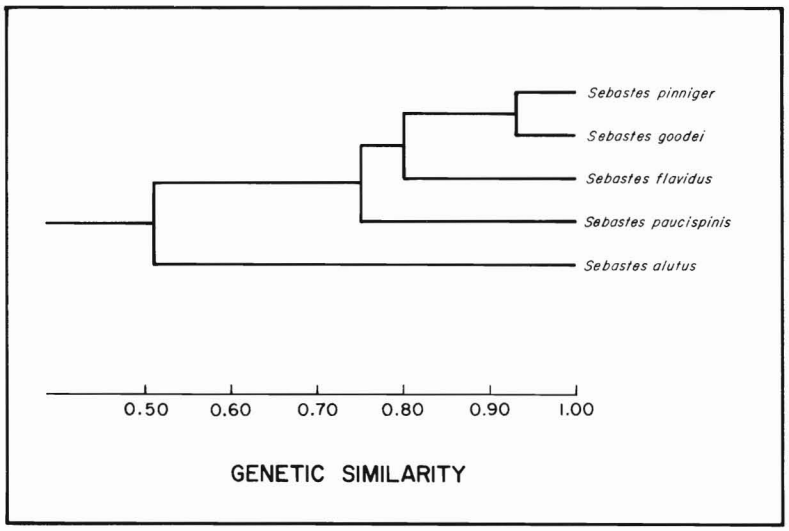

Figure 6.—Dendrogram of genetic similarities for five species of rockfish.

Although only speculative, the data presented here, therefore, suggest that chilipepper possesses the youngest lineage of the five rockfish species and may have passed through small population sizes in the recent past (in terms of evolutionary time). Canary rockfish and chilipepper are genetically quite similar and both have low levels of heterozygosity. It is possible that they have recently diverged from a common ancestor. On the other extreme, both bocaccio and Pacific ocean perch seem to be older lineages with large population sizes. As more data are accumulated on other rockfish species, the evolutionary history of the genus *Sebastes* should become clearer.

Applicability of Electrophoresis To Marine Fisheries

The effectiveness of biochemical genetic techniques as stock separation tools will vary with the species being investigated and the geographic extent of the sampling area. Generally the more variation that exists in the species, the greater the probability will be of finding systems that discriminate between subgroups of the species. However, exceptions do exist. Just one polymorphism with appropriate discriminating frequencies may be sufficient to adequately identify noninterbreeding groups or, alternatively, a species may be highly polymorphic throughout its range but show no geographic differentiation. Thus, a priori, there is no way to determine whether one species will show local differentiation or be genetically homogenous throughout the sampling range, and each species must be screened independently to determine its population structure.

Genetic differences between groups of one species are dependent on the amount of gene flow between these groups. High amounts of gene flow lead to reduced genetic differentiation. Large spawning aggregations and pelagic larvae, characteristic of many marine fishes, both contribute to high degrees of gene flow. Long migrations of individual fish may or may not contribute to high degrees of gene flow, depending on whether the migrants successfully spawn in the new areas or return to their area of origin to spawn. In marine species, such as rockfish, genetic differentiation can occur when groups consistently spawn at different areas or at different times and when the offspring of these fish return to spawn at the same time or place as their parents. Currents or gyres can act to isolate pelagic larvae and create geographic differentiation.

In marine species one would never expect to find the degree of population subdivision that is present in such anadromous species as salmonids. As more marine species are examined, the majority will likely prove to be genetically similar over broad geographic areas. In view of this limitation, subpopulations of marine species that are identified through significant differences of allelic frequencies should be managed as separate stocks. Even where dispersal during the pelagic larval period creates a situation where the adjacent stocks are genetically similar, migrations and movements of adult fish might be extremely limited. In this case, local depletion of one stock might occur in a single generation, and a substantial rebuilding period would be required before immigration of juveniles could return the stock to its former size. For this reason any morphological, growth, or fecundity differences between subgroups indicate that they should be managed as separate units even though they may be genetically homogeneous. Genetic analysis should ideally be done in conjunction with other more classical types of stock separation work, since the two approaches may identify different subgroupings of fish.

Adequate sampling in genetic studies of marine fish is a difficult problem. Ideally the sampling area should cover the total range of the species. Samples from extremes of the species ranges can often provide particularly useful comparisons. In this survey, for instance, the species' ranges are much greater than the actual sampling areas. Additionally, the samples in this survey were all collected by bottom trawling so that individuals inhabiting extremely rocky or reef areas were not sampled. Also, no samples were taken at depths of less than 40 fathoms, and no adequate test can be made of genetic differences between inshore and offshore extremes. In future studies different fishing methods may be necessary to sample the inshore component. For several of the species the data were suggestive of population differences with depth or area, but further sampling is necessary to confirm the initial

findings. Once noninterbreeding groups can be identified with confidence, the areas of contact will be especially interesting and important to resample.

Electrophoresis has been applied to a number of other fisheries problems besides population characterization. It is an extremely effective species discrimination tool and can be applied whenever the identification of a fish is in doubt. This capability can be especially important in a speciose group, such as the rockfish, where the taxonomy is not completely clear and many of the discriminating quantitative characters overlap. The potential is great for using electrophoresis in larval identification, since even very small larvae can be identified to species using electrophoresis. Additionally, electrophoresis is being used increasingly as an enforcement tool in identifying unknown or filleted individuals as to species.

Acknowledgments

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