

GENETIC VARIATION AND POPULATION STRUCTURE IN A DEEPWATER SNAPPER, *PRISTIPOMOIDES FILAMENTOSUS*, IN THE HAWAIIAN ARCHIPELAGO

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ABSTRACT

Pink snapper were collected from six different locations in the Hawaiian Archipelago and subjected to starch gel electrophoretic analysis. Of a total of 44 enzyme-coding loci screened for genetic variation, 5 polymorphic loci were detected (Adh, Gpi-A, Iddh, Ldh-C, and Umb). Each polymorphic locus exhibited two common alleles (range of individual locus heterozygosity = 0.293-0.495). The heterozygosity averaged over all 44 loci was 0.047. Observed genotype distributions at the five polymorphic loci were in general agreement with Hardy-Weinberg equilibrium expectations. However, when the collections were subdivided into two major age groups (fish about 2-5 years old vs. fish 5-14 years old), significant differences in allele frequency between groups were detected for both alcohol dehydrogenase and lactate dehydrogenase-C.

Repetitive samples in 1979 and 1980 from two localities suggested that the allele-frequency distributions were stable during the period of the study. Contingency χ^2 tests of the entire data set failed to reveal significant genetic differences among the five primary localities (Maro Reef, French Frigate Shoals, Necker, Molokai, and Hawaii) or between the two major areas (Northwestern Hawaiian Islands and main Hawaiian Islands) represented by the collections. The mean value of Wright's F_{ST} for the five polymorphic loci was 0.005 indicating little subpopulation differentiation.

The data fail to reveal significant genetic differentiation among localities. Indeed, the results are entirely consistent with the existence of a single, panmictic stock of pink snapper throughout the Hawaiian Archipelago.

The pink snapper, or opakapaka, *Pristipomoides filamentosus*, is a deepwater species found throughout the Indo-West Pacific, including South Africa, Japan, Australia, the Philippines, Samoa, and the Hawaiian Islands (Kami 1973). In the Hawaiian Islands it occurs in significant numbers from Hawaii in the southeast through Maro Reef in the northwest and is found in greatest abundance at depths of 80-150 m (Ralston 1980). For the past 15 or more years, this snapper has been the dominant species in the deep-sea handline fishery in Hawaii (Hawaii Division of Fish and Game 1960-80³; Ralston and Polovina 1982). Due largely to the developing fishery in the Northwestern Hawaiian Islands (NWHI) the annual commercial harvest of *P. filamentosus* has increased from about 33 t in 1970 to 105 t in 1980 (Hawaiian Division of Fish and Game footnote 3).

Spawning of pink snapper in Hawaii appears to be concentrated in the fall of the year, and presumed annual fecundity may be as high as 1×10^6 eggs per female (B. S. Kikkawa⁴). Fertilization in opakapaka is external and the eggs are planktonic. After hatching, the larvae remain pelagic for about 1-2 mo during which time they attain a size of 20-25 mm (J. Leis⁵). Adults are essentially demersal but virtually nothing is known about the magnitude of adult movements, either daily or seasonally.

The present genetic investigation of stock structure in *P. filamentosus* was initiated to address two questions relevant to the future management of this fishery. First, was there any detectable stock heterogeneity within the entire Hawaiian Archipelago? Second, and specifically relating to the potential impact of the emerging fishery in the NWHI on the existing fishery in the main Hawaiian Islands, was there evidence that popu-

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³Hawaii Division of Fish and Game. 1960-80. Commercial fish landings. Mimeogr., var. pag.

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lations in the NWHI were differentiated, and thus independent, from populations in the main islands?

MATERIALS AND METHODS

All specimens were obtained using commercial handline gear and were either frozen or iced at sea. Details of the collections are presented in Table 1. One series of samples (from French Frigate Shoals and Maro Reef) was filleted at sea, and the remaining carcasses (containing the tissues of interest) were preserved in an ice cold brine solution. This means of sample handling had the unfortunate effect of inactivating some of the enzymes (especially glucosephosphate isomerase and lactate dehydrogenase) so that these two enzymes could not be reliably scored in these samples. Initial screening for polymorphic loci in the pink snapper was conducted on extracts of white skeletal muscle, red skeletal muscle, heart, eye, brain, and liver. Each of these tissue samples was dissected from fresh or frozen specimens and homogenized in an equal volume of 0.1 M Tris-HCl pH 7.0 buffer (containing 1×10^{-3} M EDTA and 5×10^{-5} M NADP⁺) using a loose fitting, motorized pestle. Homogenates were centrifuged for at least 20 min at a minimum of $20,000 \times g$ (liver supernatants were routinely centrifuged a second time to minimize lipid content). The resulting supernatants were transferred to

individually labeled glass vials which were capped and stored at -75°C until the electrophoretic analysis was completed.

Electrophoresis

The supernatants were analyzed by horizontal starch gel electrophoresis (Selander et al. 1971). Each enzyme system surveyed in the initial screening for genetic variation was electrophoresed on from two to eight different buffer systems using extracts of several different tissues. Following electrophoresis, isozyme patterns were visualized using standard recipes (modified from Shaw and Prasad 1970; Selander et al. 1971; Siciliano and Shaw 1976). The umbelliferyl esterase (often called EST-D in the literature) was visualized using 4-methylumbelliferyl acetate as substrate.

Gel Scoring and Data Analysis

Patterns of enzyme variation which were consistent with the subunit structure of the homologous protein in other fishes (when known) and simple genetic models were scored and recorded as genotypes. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Commission on Biochemical Nomenclature (1973). For multilocus enzyme systems, loci were given alphabetic designations to indicate homology with known forms (e.g., Gpi-B and Ldh-C). With the exception of one very rare allele (observed once) for both ADH and UMB, each of the polymorphic enzymes screened exhibited only two detectable alleles. These two alleles are referred to hereafter by their relative electrophoretic mobility from the origin as f (= fast) and s (= slow).

Tests of Hardy-Weinberg equilibrium and calculations of average heterozygosity (H) were accomplished as described in Shaklee and Samollow (1984). A locus was considered polymorphic if the frequency of the most common allele was ≤ 0.95 .

Two types of χ^2 tests were used to test for genetic differentiation and, therefore, stock heterogeneity. First, for all polymorphic loci, contingency tests of all possible pairwise combinations of localities were conducted. Second, contingency tests comparing pooled samples representing the main Hawaiian Islands and the NWHI were conducted for all loci. Wright's F_{ST} statistic (Wright 1965, 1978) was calculated using the BIOSYS-1 computer program (Swofford and Selander 1981).

TABLE 1.—Collection details for total samples and individual collections of *Pristipomoides filamentosus* used in the electrophoretic analysis.

Collection ¹	Number	Dates	Average size ²
Maro Reef	129	Oct. 1978-Nov. 1980	584 (± 130)
a	12	Oct. 1978	
b	59	Oct. 1979	
c	9	Oct. 1979-Nov. 1980	
d	49	Nov. 1980	
French Frigate Shoals	254	Mar. 1979-May 1980	372 (± 125)*
a	27	Mar. 1979	
b	67	Oct.-Nov. 1979	
c	46	May 1980	
d	114	Nov. 1980	
Necker	127	Mar. 1979-Nov. 1980	519 (± 104)**
a	107	Mar.-May 1979	
b	20	Nov. 1979-Nov. 1980	
Kauai	25	Feb.-Apr. 1981	441 (± 90)
a	20	Feb. 1981	
b	5	Apr. 1981	
Molokai	118	Mar. 1979-Apr. 1981	333 (± 47)
a	9	Mar. 1979	
b	20	Sept. 1979	
c	5	July 1980	
d	84	Mar.-Apr. 1981	
Hawaii	63	June 1979-Apr. 1981	393 (± 55)
a	17	June-July 1979	
b	46	Mar.-Apr. 1981	

¹ See figure 1 of Shaklee and Samollow (1984) for locality information.

² Fork length, FL (± 1 standard deviation) in mm.

*FL of 38 fish from collections a and b unknown.

**FL of 79 fish from collection a unknown.

RESULTS

Forty-four presumed gene loci encoding 29 different enzymes were surveyed in a total of 716 pink snapper collected at six localities over a 2½-yr period. Thirty-nine of these loci were monomorphic in the first 100 animals screened (21 from Maro Reef, 44 from FFS, and 35 from Necker). These enzymes, which were not studied further, were aspartate aminotransferase (2 loci), acid phosphatase, adenosine deaminase, adenylate kinase, alkaline phosphatase, alanine aminotransferase (2 loci), catalase, creatine kinase (3 loci), esterase (2 loci), glyceraldehyde-3-phosphate dehydrogenase (2 loci), glutamate dehydrogenase, glucosephosphate isomerase-A, glycerol-3-phosphate dehydrogenase (2 loci), hexosediphosphatase (2 loci), isocitrate dehydrogenase, lipoamide dehydrogenase (= diaphorase), lactate dehydrogenase (A and B), malate dehydrogenase (2 loci), malate dehydrogenase-NADP⁺ (= malic enzyme), mannosephosphate isomerase, peptidase (4 loci), peroxidase, phosphogluconate dehydrogenase, phosphoglucomutase, pyruvate kinase, superoxide dismutase, and xanthine

dehydrogenase. The remaining five loci were polymorphic and the conditions for their analysis are summarized in Table 2. Since only 5 out of 44 loci in pink snapper were found to be polymorphic, $P_{.95}$ is estimated to be 0.114 in this species.

Zymogram patterns showing the commonly observed phenotypes for 4 of the 5 polymorphic enzymes in pink snapper are shown in Figure 1. The

TABLE 2.—Polymorphic enzymes in pink snapper, *Pristipomoides filamentosus*: Characteristics and conditions for analysis.

Enzyme (EC number)	Locus	Subunit structure ¹	Tissue	Buffer
Alcohol dehydrogenase (1.1.1.1)	Adh	dimer	liver	EBT ²
Glucosephosphate isomerase (5.3.1.9)	Gpi-B	dimer	muscle	CAEA ³
L-iditol dehydrogenase (1.1.1.14)	Iddh	tetramer	liver	TC-1 ⁴
Lactate dehydrogenase (1.1.1.27)	Ldh-C	tetramer	eye	TC-5 ⁵
Umbelliferyl esterase (3.1.1.-)	Umb	dimer	liver	EBT

¹ Presumed structure based on isozyme banding pattern in heterozygotes (see text).

² EDTA-boric acid-Tris pH 8.6 buffer of Boyer et al. (1963).

³ Citric acid-aminopropyl-diethanolamine (gel = pH 7.5, electrodes = pH 7.2) buffer of Clayton and Tretiak (1972).

⁴ Tris-citric acid pH 7.0 buffer (buffer 1 of Shaw and Prasad 1970).

⁵ Tris-citric acid pH 7.0 buffer (same as TC-1 but gel buffer is a 1:6.5 dilution of the electrode buffer).

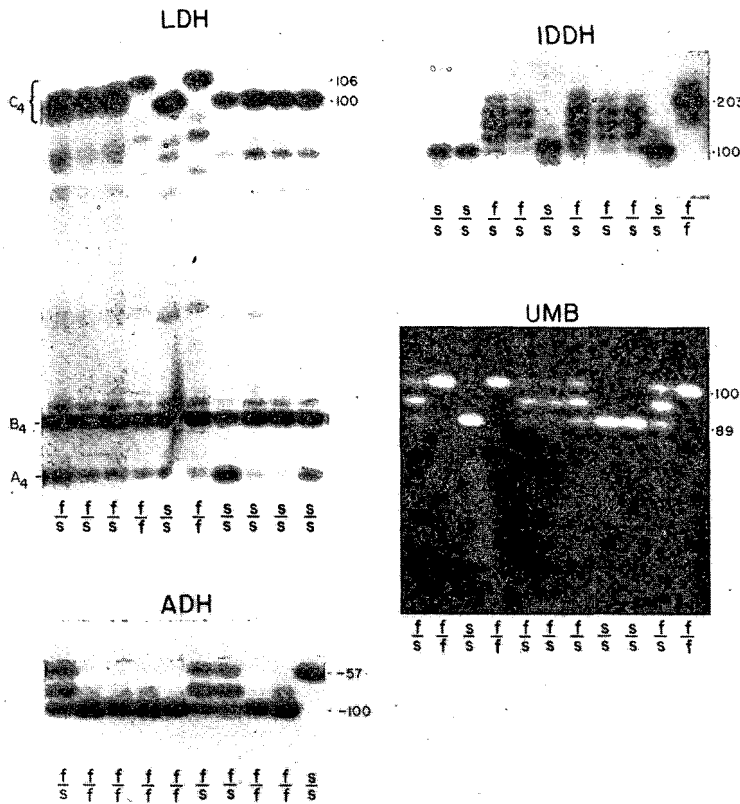


FIGURE 1.—Isozyme patterns of pink snapper, *Pristipomoides filamentosus*. LDH = lactate dehydrogenase, IDDH = L-iditol dehydrogenase (= sorbitol dehydrogenase), ADH = alcohol dehydrogenase, UMB = umbelliferyl esterase. Alleles are indicated at the right of each gel. Scoring of individual genotypes (by allelic class) is indicated at the bottom of each gel. Note that the sample origin is at the bottom and the anode is toward the top except for ADH where the origin is at the top (and the cathode is toward the bottom).

allele frequencies, heterozygosity per locus, F_{ST} per locus, and the number of genes successfully scored for each polymorphic locus at each locality are presented in Table 3. Glucosephosphate isomerase-B (Gpi-B), lactate dehydrogenase-C (Ldh-C), and L-iditol dehydrogenase (Iddh) (= sorbitol dehydrogenase) each exhibited only the two alleles shown in Figure 1 and Table 3. Alcohol dehydrogenase (Adh) exhibited one additional allele ("very slow") in one heterozygote. This rare allele was pooled with the "slow" allele in the analysis. Similarly, umbelliferyl esterase (Umb) exhibited one additional allele ("very slow") in one heterozygote. This rare allele was pooled with the "slow" allele in the analysis. χ^2 tests of goodness of fit to Hardy-Weinberg expectations of genotype distributions revealed two significant deviations (out of a total of 25 tests). These were a heterozygote deficiency for Ldh-C at Necker Island ($\chi^2_1 = 8.17$; $P < 0.005$), and a heterozygote excess for Umb at Hawaii ($\chi^2_1 = 3.95$; $P < 0.05$). Since one significant outcome in 20 tests is expected by chance given an $\alpha = 0.05$, this is not surprising. The per locus heterozygosity ranged from 0.293 for the Gpi-B locus to 0.495 for the Umb locus. The average heterozygosity across all loci (H) was 0.047.

A comparison of the allele frequencies at each locus across the five localities (Table 3) revealed that there was little overall variation among locations. χ^2 contingency tests of all possible pairwise comparisons of localities (10 comparisons \times 5 loci = 50 tests) yielded only one significant value, that for Iddh, Necker Island vs. Molokai ($\chi^2_1 = 5.99$; $P < 0.025$). The apparent homogeneity was even more evident in χ^2 tests of the two pooled groups (NWHI vs. main) where no significant outcomes occurred in the five tests. Three other measures of genetic characteristics of these samples also failed to reveal subpopulation differentiation. The number of common alleles per polymorphic locus was two at all localities for all enzymes. The average heterozygosity per locus (H) showed almost no difference (Maro Reef $H = 0.045$, French Frigate Shoals $H = 0.047$, Necker Island $H = 0.048$, Molokai $H = 0.047$, Hawaii $H = 0.046$) among localities. Finally, the values of Wright's F_{ST} were small for all five polymorphic loci and the mean F_{ST} was only 0.005. Overall, none of the measures used indicated subpopulation or stock heterogeneity in this species in the Hawaiian Islands.

It could reasonably be argued that the above analyses of pooled collections consisting of fish of

TABLE 3.—Allele frequencies, heterozygosity values, and F_{ST} values at five polymorphic loci in pink snapper, *Pristipomoides filamentosus*. Localities as in figure 1 of Shaklee and Samollow (1984).

Locus ¹ (heterozygosity)	Alleles ²	Locality						
		Maro Reef	FFS	Necker	Molokai	Hawaii	NWHI ³	Main ⁴
Adh ($h = 0.456$) $F_{ST} = 0.004$	f	0.673	0.679	0.607	0.638	0.611	0.657	0.632
	s	0.327	0.321	0.393	0.362	0.389	0.343	0.368
	(n/N)	(220/ 119)	(346/ 187)	(224/ 117)	(232/ 116)	(126/ 63)	(790/ 423)	(408/ 204)
Gpi-B ($h = 0.287$) $F_{ST} = 0.001$	f	0.164	0.180	0.154	0.179	0.155	0.171	0.177
	s	0.836	0.820	0.846	0.821	0.845	0.829	0.823
	(n/N)	(140/ 70)	(316/ 160)	(104/ 52)	(212/ 107)	(110/ 55)	(560/ 282)	(372/ 187)
Iddh ($h = 0.431$) $F_{ST} = 0.006$	f	0.314	0.301	0.410	0.262	0.336	0.325	0.299
	s	0.686	0.699	0.590	0.738	0.664	0.675	0.701
	(n/N)	(210/ 119)	(186/ 96)	(78/ 42)	(214/ 107)	(110/ 55)	(474/ 257)	(374/ 187)
Ldh-C ($h = 0.379$) $F_{ST} = 0.011$	f	0.209	0.257	0.232	0.307	0.236	0.243	0.273
	s	0.791	0.743	0.768	0.693	0.764	0.757	0.727
	(n/N)	(110/ 58)	(358/ 185)	(142/ 78)	(228/ 116)	(110/ 55)	(610/ 321)	(388/ 196)
Umb ($h = 0.496$) $F_{ST} = 0.003$	f	0.571	0.502	0.572	0.582	0.563	0.537	0.556
	s	0.429	0.498	0.428	0.418	0.437	0.463	0.444
	(n/N)	(254/ 129)	(494/ 254)	(250/ 127)	(232/ 116)	(126/ 63)	(998/ 512)	(408/ 204)

¹Adh = alcohol dehydrogenase, Gpi = glucosephosphate isomerase, Iddh = L-iditol dehydrogenase (= sorbitol dehydrogenase), Ldh = lactate dehydrogenase, Umb = umbelliferyl esterase, h = per locus heterozygosity, F_{ST} = Wright's (1965) fixation index.

²f = fast, s = slow, n = number of genes successfully scored, N = number of individuals analyzed.

³Consisting of fish from Maro Reef, French Frigate Shoals (FFS), and Necker Island.

⁴Consisting of fish from Molokai, Hawaii, and 25 specimens from Kauai.

very different sizes (and therefore different ages) taken over a considerable period of time may have obscured important information and possible heterogeneity in the data. This is particularly true since the pink snapper were collected over a 2½-yr period and ranged in size from 230 to 770 mm FL (fork length)—presumably representing a range of ages from 2 to over 16 yr (Ralston and Miyamoto 1983). With this in mind, the data set was partitioned (where fish lengths were known and sample sizes were large enough to allow reasonable statistical tests) to allow analyses of the collection for 1) evidence of variation in allele frequency between age classes, 2) evidence of year-to-year variability in allele frequency at a location, and 3) evidence of allele frequency variation within a homogeneous age class among localities. The samples from Maro Reef and French Frigate Shoals (FFS) allowed partitioning with regard to size classes. The frequency distributions characterizing individual collections making up the samples for Maro Reef and FFS as well as those for the three unpartitioned areas (Necker, Molokai, and Hawaii) are shown in Figure 2. Because the collections from both Maro Reef and FFS contained adequate numbers of specimens throughout the size range, they were subdivided into two groups. One group consisting of small, young fish (300-500 mm FL; about ages 2-5 yr—see Ralston and Miyamoto 1983) and one of large, old fish (501-770 mm FL; about ages 5-16+ yr). The allele-frequency distributions characteristic of these separate groups are shown in Table 4. For Maro Reef and FFS, χ^2 analysis of the data for goodness-of-fit to Hardy-Weinberg expectations revealed no significant deviations (5 loci \times 2 locations \times 2 size groups = 20 tests), although it should be emphasized that the statistical test is not robust for small sample sizes (Fairbairn and Roff 1980). Heterogeneity χ^2 tests between size classes at both Maro Reef and FFS revealed only one statistically significant difference (for Adh at Maro Reef, $\chi^2_1 = 4.72$; $P < 0.05$). A third test for changes in allele frequency with size was conducted on a pooled data set including all fish of known fork length from Maro Reef, FFS, and Necker. This pooled NWHI sample had considerably larger sample sizes in each cell making the statistical tests more robust. Genotype proportions at all five loci in both size (age) groups in the combined NWHI samples were in agreement with Hardy-Weinberg expectations. Of the five contingency χ^2 tests between size (age) groups, only that for Ldh-C was significant ($\chi^2_1 = 4.22$; $P <$

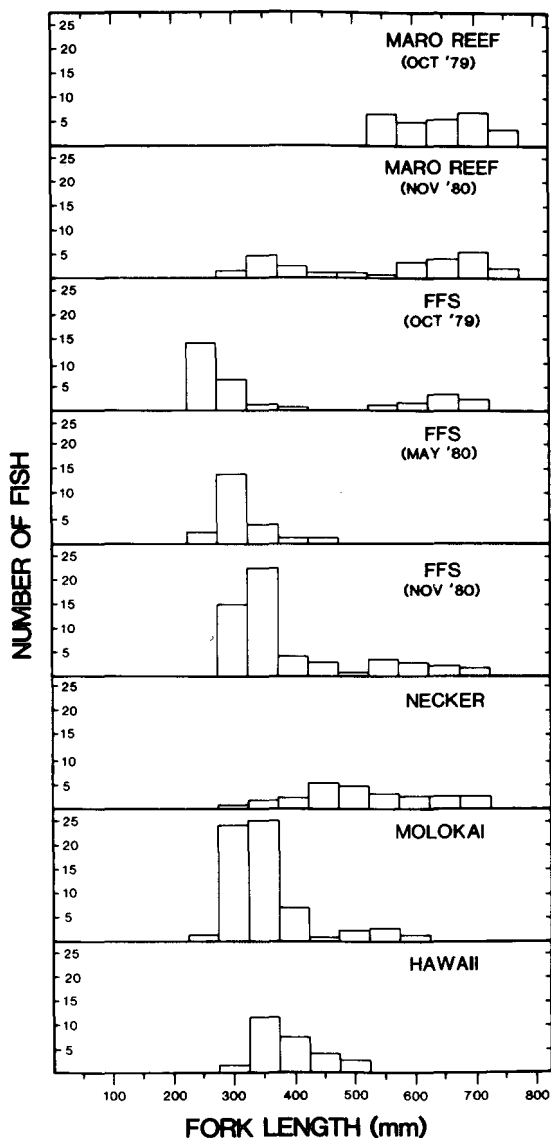


FIGURE 2.—Size-frequency histograms for eight snapper collections.

0.05). This significant outcome was due to an increase in the frequency of the more common (slow) allele with increased size (age). The increase in the frequency of the slow allele with increasing size was characteristic of the individual samples from both Maro Reef and FFS. A similar trend in allele-frequency change, this one involving an increase in the frequency of the rarer (slow) allele of Adh, was observed in the samples. Although the trend for Adh was statistically significant in the Maro Reef sample, it was not in the pooled NWHI

TABLE 4.—Frequencies of the more abundant allele at each polymorphic locus in pink snapper of different sizes (ages).

Locus ¹	Allele ¹	Maro Reef		FFS		NWHI ²	
		300-500 mm	501-770 mm	300-500 mm	501-770 mm	300-500 mm	501-770 mm
Adh	f	0.800 (N) (25)	0.635 (78)	0.697 (117)	0.639 (18)	0.702 (161)	0.624 (117)
Gpi-A	s	0.846 (N) (26)	0.845 (42)	0.833 (120)	0.816 (19)	0.830 (171)	0.847 (85)
Iddh	s	0.708 (N) (24)	0.676 (74)	0.687 (75)	0.833 (3)	0.695 (118)	0.649 (97)
Ldh-C	s	0.740 (N) (25)	0.821 (28)	0.731 (117)	0.816 (19)	0.735 (149)	0.833 (54)
Umb	f	0.654 (N) (26)	0.564 (94)	0.476 (125)	0.500 (36)	0.514 (176)	0.555 (155)

¹Abbreviations of loci and alleles as in Table 3; N = number of fish successfully scored.

²Consisting of fish from Maro Reef and French Frigate Shoals (FFS) and fish from Necker with known fork length.

sample. The other three loci exhibited no such clear trends in changing allele frequency with size (age). These analyses indicate that allele frequencies at some loci (Adh and Ldh-C) in the opakapaka may exhibit important changes related to size (age) and caution against an uncritical analysis of pooled data.

It was possible to analyze temporally subdivided samples from Maro Reef and FFS to see whether or not significant changes in allele frequency at a location were occurring through time. The allele frequencies characteristic of these temporal samples are shown in Table 5. Unfortunately, in order to obtain adequate sample sizes it was necessary to include specimens of all sizes (ages) in these samples. Of the 19 sets, only Umb in the May 1980 sample from FFS was significantly out of Hardy-Weinberg equilibrium ($\chi^2_1 = 6.14$; $P < 0.025$), exhibiting an excess of heterozygotes. Of the 10 contingency χ^2 tests between successive samples, only that for Ad at Maro Reef was statistically significant ($\chi^2_1 = 5.80$; $P < 0.025$) and that in-

TABLE 5.—Frequencies of the more abundant allele at each polymorphic locus in pink snapper collected at different times. — = no data because tissues had been stored in brine (see Materials and Methods).

Locus ¹	Allele ¹	Maro Reef		French Frigate Shoals		
		Oct. 1979	Nov. 1980	Oct. 1979	May 1980	Nov. 1980
Adh	f	0.593 (N) (54)	0.750 (50)	—	0.656 (45)	0.693 (109)
Gpi-A	s	—	0.827 (N) (52)	—	0.807 (44)	0.825 (114)
Iddh	s	0.696 (N) (51)	0.677 (43)	—	0.656 (45)	0.740 (48)
Ldh-C	s	—	0.796 (N) (49)	—	0.733 (43)	0.752 (113)
Umb	f	0.568 (N) (59)	0.590 (50)	0.433 (67)	0.478 (45)	0.540 (112)

¹Abbreviations of loci and alleles as in Table 3; N = number of fish successfully scored.

involved fish of significantly different sizes in the two samples (Fig. 2). Although this temporal analysis is not as complete as one might wish, it does suggest that allele frequencies were reasonably constant over the 2½ yr period of the present study.

Given that allele frequencies remained relatively constant through time but exhibited significant changes (at two loci) associated with fish size (age), a final analysis of the data was conducted. This involved fish from all five locations but only fish of 300-550 mm FL (about 2-6 yr old; see Ralston and Miyamoto 1983). The allele frequencies characteristic of these samples are presented in Table 6. In all cases (5 loci × 5 locations = 25 tests) the data were in agreement with Hardy-Weinberg equilibrium expectations. Even more importantly, the outcomes of all five χ^2 contingency tests (involving all locations) were non-significant. The results reinforce the earlier conclusions that no among-locality genetic differentiation exists in pink snapper throughout the

TABLE 6.—Frequencies of the more abundant allele at each polymorphic locus in pink snapper between 300 and 550 mm FL.

Locus ¹	Allele ¹	Location						
		Maro Reef	FFS	Necker	Molokai ²	Hawaii	NWHI ³	Main ⁴
Adh	f	0.759 (N) (29)	0.696 (120)	0.542 (24)	0.658 (101)	0.630 (54)	0.688 (173)	0.645 (155)
Gpi-A	s	0.850 (N) (30)	0.837 (123)	0.806 (31)	0.815 (100)	0.852 (54)	0.832 (184)	0.831 (154)
Iddh	s	0.722 (N) (27)	0.687 (75)	0.667 (24)	0.723 (101)	0.657 (54)	0.698 (126)	0.697 (155)
Ldh-C	s	0.750 (N) (26)	0.729 (120)	0.833 (9)	0.715 (100)	0.769 (54)	0.735 (155)	0.740 (154)
Umb	f	0.667 (N) (33)	0.480 (128)	0.581 (31)	0.569 (101)	0.565 (54)	0.526 (192)	0.568 (155)

¹Abbreviations of loci and alleles as in Table 3; N = number of fish successfully scored.

²Including 25 fish from Kauai.

³Consisting of fish from Maro Reef, French Frigate Shoals, and Necker.

⁴Consisting of fish from Kauai, Molokai, and Hawaii.

Hawaiian Islands. Absolutely no evidence of multiple stocks was found.

DISCUSSION

Genetic Inferences

Because it was not possible in the present study to verify the Mendelian nature of the observed variation in enzyme phenotypes in *Pristipomoides filamentosus* by direct genetic tests, such a genetic basis can only be inferred from the results of the χ^2 goodness-of-fit tests (to Hardy-Weinberg expectations) and by comparison of the snapper data with genetic (Purdom et al. 1976; May et al. 1979; Kornfield et al. 1981), molecular (Darnall and Klotz 1975; Mo et al. 1975), and electrophoretic data (Engel et al. 1971) for these same enzymes in other fish species. The genetic and molecular bases of four of the enzymes scored for variation in *P. filamentosus* have been well documented in other fish species. For three of these (ADH, GPI, and IDDH) the banding patterns observed in presumed heterozygote snappers were entirely consistent with expectations from other studies. However, the results for two enzymes require additional comment.

Lactate Dehydrogenase-C

Three distinct Ldh loci characterize diploid bony fishes (Markert et al. 1975). The Ldh-C locus typically encodes the eye-specific LDH isozyme (Whitt 1969, 1970; Shaklee et al. 1973), but in some groups it encodes an isozyme predominant in liver (Sensabaugh and Kaplan 1972; Shaklee et al. 1973). Both the eye-specific and the liver-predominant LDH isozymes have been characterized biochemically (Whitt 1970; Sensabaugh and Kaplan 1972). Electrophoretic variants of the eye-specific LDH encoded at the Ldh-C locus have been shown to be inherited in Mendelian fashion in freshwater sunfish (Whitt et al. 1971) and poeciliids (Leslie and Vrijenhoek 1977; Morizot and Siciliano 1979). The Ldh-C locus has been reported to be variable in numerous species. In essentially all of these cases, the isozyme pattern of the heterozygote has been difficult to resolve into the expected five isozymes, rather it typically appears as a smear of LDH activity extending from the region of the slow allele homozygote to the region of the fast allele homozygote. The pattern of LDH-C variation in *P. filamentosus* shown in Figure 1 is similar to that reported for these

other species. Although we were unable to resolve the theoretically predicted five C subunit-containing bands in presumed heterozygotes, their status as heterozygotes seems secure since these individuals exhibited two bands at the position of the B_3C_1 heterotetrameric isozyme (one having the same mobility as the single slow $B_3C_1^3$ heterotetramer in the slow homozygotes and one having the same mobility as the single fast $B_3C_1^1$ heterotetramer in the fast homozygotes), just as expected for such heterozygotes (Fig. 1).

Umbelliferyl Esterase

Although there are numerous published studies of biochemical properties, genetic variation, and inheritance in fish esterases (Koehn 1969; Fujino 1970; Holmes and Whitt 1970; Metcalf et al. 1972; Smith et al. 1978; Leslie and Pontier 1980; Van Beneden et al. 1981), the diversity and heterogeneity of these enzymes makes the assignment of homology difficult. However, since virtually all of the above investigations involved the use of α -naphthyl esters as substrates in the staining reaction and the UMB of *P. filamentosus* (stained using 4-methylumbelliferyl acetate as substrate) does not show detectable activity with α -naphthyl acetate, it seems unlikely that the umbelliferase of the pink snapper is homologous with any of these esterases of other fishes. The only other reports of UMB variation in fish are those of Ward and Beardmore (1977) (= "Est-D") for plaice and of Shaklee et al. (1983) for blue marlin. In both of these species, as in *P. filamentosus*, UMB exhibits single-banded homozygotes and triple-banded heterozygotes (with isozyme staining intensities in an approximate 1:2:1 ratio as expected for a dimeric protein). These observations and the general agreement between observed genotype distributions and those expected, assuming Hardy-Weinberg equilibrium, support our genetic interpretations of the observed variation in UMB in pink snapper.

Stock Structure

Significant differences in allele frequency among fish of differing ages within a single Mendelian population or stock can be due to 1) the differential mortality of certain genotypes (natural selection), 2) genotype-specific differences in catchability (distribution, activity, behavior, etc.), 3) chance fluctuations due to nonrepresentative spawning in different years (genetic

drift) or, 4) some combination of the above. Unfortunately, the short duration of most electrophoretic studies and/or inadequate sample sizes for various age classes often prevent or limit investigations of such age-dependent changes in allele frequency. No year class heterogeneity in transferrin allele frequencies was detected in 27 consecutive year classes of Atlantic cod by Jamieson (1975). Similarly, no year class variation was reported for mackerel EST by Smith et al. (1981). On the other hand, changing patterns of allele-frequency distribution with increasing age (size) have been noted in several fish species, including the blenny, *Anoplarchus purpureus* (Johnson 1971), the eelpout, *Zoarces viviparus* (Christiansen et al. 1974), the mummichog, *Fundulus heteroclitus* (Mitton and Koehn 1975), the plaice, *Pleuronectes platessa* (Beardmore and Ward 1977), and several New Zealand commercial fishes (Smith 1979a; Gauldie and Johnston 1980). In many of the latter cases, it has been suggested that natural selection is the underlying cause of the shift in allele frequencies with age. Our analysis of the pink snapper data (Table 4) revealed that both alcohol dehydrogenase and Ldh-C exhibited significant differences in allele frequency between young and old fish. However, we do not have any direct information that these changes are due to natural selection.

There have been few electrophoretic studies of commercial fishes which have addressed the question of temporal stability of allele frequencies. In the American eel, *Anguilla rostrata*, data for 4 successive years revealed that patterns of genetic differentiation were generally unchanged through time (Koehn and Williams 1978). On the other hand, studies of pink salmon (Aspinwall 1974) have demonstrated substantial temporal heterogeneity in allele frequencies due to the existence of distinct spawning stocks isolated in time. Temporal differences in gene frequencies related to different spawning stocks have also been suggested by Kornfield et al. (1982) for Atlantic herring. The present analysis of opakapaka allele frequencies through time (Table 5) revealed no temporal differences.

There can be no doubt that the Hawaiian Islands offer a sharply discontinuous distribution of adult habitats for *Pristipomoides filamentosus* given that the species is almost completely restricted to water depths of 50-200 m in Hawaii (Ralston 1980) (200 m contour lines in figure 1 of Shaklee and Samollow 1984). Furthermore, there is no reason to believe that adult pink snapper—

strictly demersal fish—migrate through the open, oceanic waters between islands. It is therefore somewhat surprising that no evidence of genetic subdivision of opakapaka among localities within the Archipelago was observed. This seems particularly surprising given that there are several reports of stock heterogeneity in other demersal marine fishes such as Atlantic cod (Jamieson 1975; Cross and Payne 1978; Jamieson and Turner 1978), walleye pollock (Grant and Utter 1980), New Zealand snapper (Smith et al. 1978), and two species of flatfishes (Fairbairn 1981a, b). The genetic homogeneity observed for pink snapper in the present study would seem a singular exception when compared with the above results were it not for the fact that numerous other demersal species exhibit similar patterns of apparent genetic homogeneity, e.g., plaice (Purdom et al. 1976; Ward and Beardmore 1977), hake (Smith et al. 1979), ling (Smith 1979b; Smith and Francis 1982), and New Zealand hoki (Smith et al. 1981). However, it must be remembered that the embryonic and larval stages of *P. filamentosus* and most, if not all, of these other species are pelagic and, therefore, serve as a dispersal phase. Given that this pelagic stage apparently lasts for 1-2 mo in pink snapper, it would not seem unreasonable that larval dispersal among localities due to wind-driven currents within the Hawaiian Islands would be of sufficient magnitude to ensure adequate gene flow among adult populations to prevent detectable genetic differentiation (Lewontin 1974).

If this interpretation is correct, it would mean that although the total pink snapper harvest in Hawaii is derived from numerous small geographically separated fisheries, each associated with discontinuous patches of suitable habitat (islands, reefs, and banks), the pink snappers themselves are all members of a single, large panmictic population distributed throughout the Hawaiian Archipelago. Based on the above information, it seems appropriate to manage the pink snapper fishery in Hawaii as a single unit stock—including both the main Hawaiian Islands and NWHI. However, since the observed genetic homogeneity does not unambiguously establish the existence of a unit stock (it is merely consistent with this hypothesis), this management policy must remain open to reevaluation in the future especially if data of another kind should suggest stock heterogeneity.

Genetic aspects of population structure have now been studied in four marine species throughout the Hawaiian Archipelago. In three of these

species—a spiny lobster, *Panulirus marginatus* (Shaklee and Samollow 1984), a damselfish, *Stegastes fasciolatus* (Shaklee 1984), and the pink snapper discussed in the present report—no evidence of subpopulation or stock heterogeneity was found. This lack of demonstrable population subdivision occurs in spite of the fact that 1) a range of over 2,400 km in length was sampled, 2) adult habitats for all three species are clearly discontinuous and, in places, separated from one another by extensive, deep-water expanses, 3) one of the species (*Panulirus marginatus*) is endemic to the Hawaiian Islands (implying a somewhat limited dispersal ability), and 4) one species (*Stegastes fasciolatus*) has demersal eggs, a relatively short-lived pelagic larval stage (possibly as short as 20 d), and is territorial as an adult. That not all marine organisms in the Hawaiian Archipelago are characterized by single, large, panmictic populations is dramatically illustrated by the fourth species, a limpet *Cellana exarata*. This species exhibits extensive population subdivision both between the NWHI and the main Hawaiian Islands and within each of these major areas (Shaklee and Samollow 1980; Shaklee 1983; Samollow 1984⁶). Together, the patterns of population structure exhibited by these four species in the Hawaiian Islands provide some insight into the extent and pattern of subpopulation differentiation in marine organisms in an archipelagic system. However, it seems apparent that considerably more investigation will be necessary before a comprehensive picture of patterns of subpopulation differentiation, not to mention an understanding of their underlying bases, becomes clear.

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