Abstract.– Spiny lobsters *Panulirus marginatus* from the Hawaiian Archipelago have undergone heavy fishing pressure since 1980 and have experienced increases in asymptotic length and decreases in mean length at onset of egg production. Prior to the expansion of the fishery, allozyme variation was surveyed, and populations were found to be polymorphic at seven loci. In 1987 we collected spiny lobsters from two of the previously sampled locations (Necker Island and Maro Reef) to test for significant changes in population structure or level of genetic variability. No significant changes through time were found at five of the seven loci. Significant differences were detected at *Est3* and *EstD*; however, frequencies at esterase loci have been previously shown to fluctuate through time. Observed number of allelic classes and observed heterozygosities have remained unchanged. The data suggest that no change in the amount of variability has occurred in spiny lobster since the expansion of the fishery.

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Genetic Variation in Highly Exploited Spiny Lobster *Panulirus marginatus* Populations from the Hawaiian Archipelago

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Commercial trapping for the spiny lobster *Panulirus marginatus* in the northwestern Hawaiian Islands began in 1976 shortly after research cruises documented the resource in abundance. Two banks, Necker Island and Maro Reef, have been most heavily fished since the beginning of the fishery. In 1986 and 1987 research cruises repeated the earlier sampling at Necker Island and Maro Reef by trapping at the same sites on each bank with the same gear to document any changes that had occurred in density, population, and genetic parameters (Polovina 1989). The repeat sampling documented substantially reduced lobster densities as indicated by catch rates, which in 1986–87 were 37% and 68% of their 1977 levels at Necker Island and Maro Reef, respectively (Polovina 1989). A statistically significant ($P<0.05$) increase in the asymptotic lengths and decreases in the mean lengths at onset of egg production occurred between the 1977 and 1986–87 sampling at both locations (Polovina 1989).

While changes in these population parameters could be due to density-dependence relationships, some type of genetic change also may have occurred from selection induced by the fishing pressure (Nelson and Soulé 1987). For example, the fishery harvests lobsters at about the size the females first begin producing eggs. It is estimated that spiny lobster enter the fishery at 3.1 years of age and that the mean age females begin producing eggs is 2.5 years (Polovina 1989). Thus, heavy fishing pressure may select for female lobsters that sexually mature at a smaller size. Reduction in the total amount of genetic diversity or changes in the stock structure of the exploited populations are also potential results of intense fishing pressure (Allendorf et al. 1987). These latter types of genetic changes can often be monitored through allozyme analyses (Nelson and Soulé 1987), and are the subject of this paper.

Spiny lobsters from seven localities in the Hawaiian Archipelago were collected between 1978 and 1980, prior to the expansion of the commercial fishery, and analyzed for allozyme variation by Shaklee and Samollow (1984). Although overall levels of variation were quite low, they observed polymorphism at 7 of the 46 loci examined. However, no clear pattern of genetic differentia-

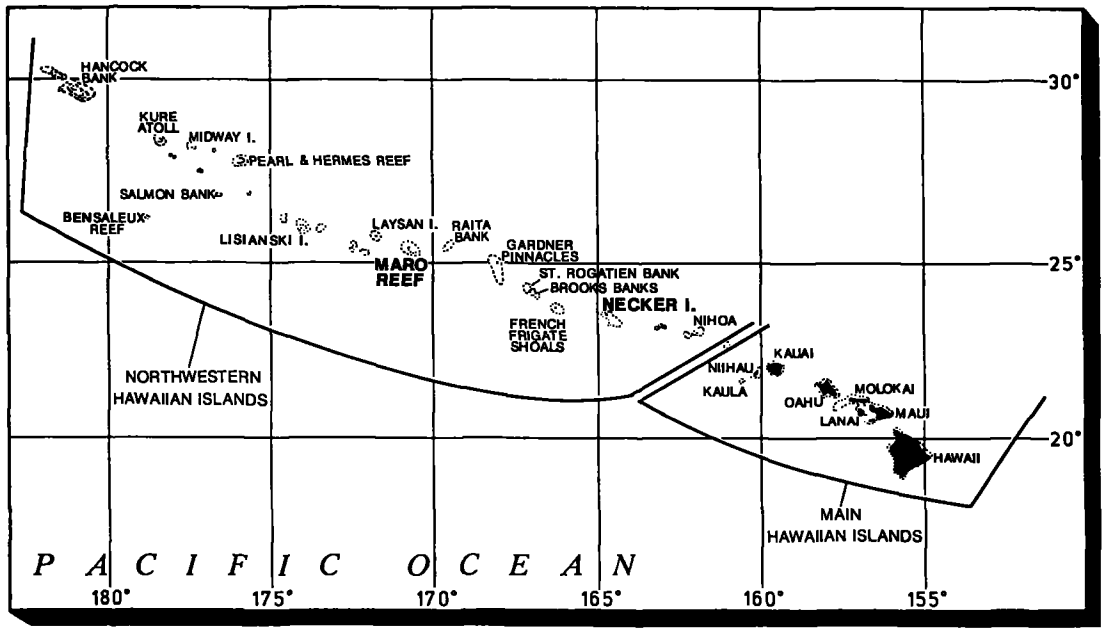

Figure 1
Location of Maro Reef and Necker Island within the Hawaiian Archipelago.

tion among populations was detected, and they concluded that a single panmictic stock of spiny lobster exists throughout the Hawaiian Archipelago.

Our objective was to reexamine allozyme variation at those seven loci to determine if fishing pressure since 1980 affected population structure or genetic variability. During 1987, new collections of spiny lobsters from two of the same localities in the northwestern Hawaiian Islands (Maro Reef and Necker Island) were made. These two sites represent the mainstay of the commercial fishery. They are the most productive banks, have been fished the longest, and annually receive the greatest fishing pressure.

Analysis showed that the observed number of allelic classes and observed heterozygosity have remained essentially unchanged between 1978 and 1987. These data provide no evidence that population bottlenecks have occurred. Fishing pressure since 1980 has not reduced the genetic variability as detected by protein electrophoresis in spiny lobster from either of these two sites in the northwestern Hawaiian Islands.

Methods and materials

Muscle and digestive gland from 200 specimens from Maro Reef and Necker Island (Fig. 1) were collected, frozen onboard ship, and shipped frozen to the laboratory for analysis. Upon receipt, tissues were subsampled for electrophoresis and stored at −80°C for up to 4 weeks while analysis was completed.

Electrophoretic methods generally followed May et al. (1979) and Shaklee and Samollow (1984) to facilitate comparison. Samples were analyzed for the products of seven loci previously identified as polymorphic: *Est3*, Esterase, E.C. 3.1.1.–; *EstD* (*Umb*), Esterase, E.C. 3.1.–.–, resolved with 4-methylumbelliferyl acetate; *Gpi*, glucosephosphate-6-isomerase, E.C. 5.3.1.9; *Mpi*, mannose-6-phosphate isomerase, E.C. 5.3.1.8; *Pgm*, phosphoglucomutase, E.C. 5.4.4.2; *Tpep1,2* (*Pep-1,2*), tripeptidase aminopeptidase, E.C. 3.4.–.–, resolved with L-leucyl-L-leucyl-L-leucine. However, in contrast to the methods of Shaklee and Samollow (1984) who used horizontal and vertical gels, all analyses were performed on horizontal starch gels. *Tpep1* was resolved on an additional buffer, amine-citrate gel and tray buffer, pH 6.9 (Clayton and Tretiak 1972).

The electrophoretic data were analyzed using BIOSYS-1 (Swofford and Selander 1981). Allelic classes followed those of Shaklee and Samollow (1984), who pooled alleles into f(fast), m(medium), and s(slow) classes for numerical analyses. Pooling was required in their study and ours because many alleles were too rare to include in the statistical tests. Actual mobilities observed were also recorded.

Comparisons between the 1978–80 and 1987 data sets for each reef were performed using a chi-square contingency analysis based on the number observed in

Table 1

Allele frequencies for seven polymorphic loci in the spiny lobster *Panulirus marginatus*. Data from 1978–80 are those of Shaklee and Samollow (1984). Alleles are identified by relative mobility classes assigned by Shaklee and Samollow (1984). Actual mobilities observed in this study are also given. Numbers of individuals successfully scored are given in parenthesis.

	Est3			*EstD*			*Gpi*			
Location and date	f	m 100	s 93	f	m 100	s	f 100	m 88	s 66	ff 145
Maro Reef										
1978–80	0.003	0.957 (386)	0.040	0.001	0.988 (340)	0.010	0.959	0.039 (320)	0.002	—
1987	—	0.968 (174)	0.032	—	1.000 (199)	—	0.970	— (198)	0.028	0.003
Necker Island										
1978–80	—	0.971 (666)	0.030	0.011	0.980 (533)	0.008	0.966	0.033 (535)	0.001	—
1987	—	0.992 (194)	0.008	—	1.000 (200)	—	0.955	0.002 (200)	0.020	0.022

	Mpi		*Pgm*			*Tpep1*		*Tpep2*		
	f 101	f+s 96, 100	f 200	m 100	s −33 −66	f 100	s 66	f ***	m 100	s 89
Maro Reef										
1978–80	0.011 (418)	0.989	0.007	0.984 (345)	0.009	0.725 (142)	0.275	—	0.988 (335)	0.012
1987	0.005 (193)	0.995	0.003	0.977 (198)	0.020	0.768 (179)	0.232	0.006	0.975 (180)	0.019
Necker Island										
1978–80	0.007 (649)	0.993	0.007	0.976 (558)	0.017	0.785 (142)	0.215	0.001	0.984 (425)	0.015
1987	0.003 (188)	0.998	—	0.985 (200)	0.014	0.768 (144)	0.232	—	0.980 (176)	0.020

each allelic class. Shaklee and Samollow (1984) found no heterogeneity between year classes for any loci except *EstD*, and thus they report pooled frequencies. They provide annual frequency estimates for *EstD* for 1979 and 1980.

We also compared the 1987 data sets from Maro Reef and Necker Island using chi-square contingency analysis.

Results

Allozyme analysis

All loci previously observed to be polymorphic were also found to be variable in the new samples, with the exception of *EstD* (Table 1). Allele classes observed by Shaklee and Samollow (1984) and not observed in this study were *Est3(f)*, *EstD(f)*, and *EstD(s)*. We observed *Gpi(145)* in both populations; this allele was not previously reported. Additionally, when analyzing *Tpep1* allozymes, we detected a *Tpep3(94)* allele in a single individual from Maro Reef which was not observed previously. Isozyme patterns agreed with descriptions provided in the earlier study. There were no indications of non-genetic variation at the esterase or any of the other loci.

Genotypic frequencies at all loci fit the expectation of Hardy-Weinberg equilibrium with the exception of *Est3* in Maro Reef ($P = 0.041$) and *Mpi* in both populations. *Mpi* has an unusual distribution of alleles between the two sexes (Shaklee 1983). Males always carry at least one slow allele, while females rarely have this variant. The common and slow variant alleles were pooled for the statistical analyses, and only the frequency of the fast allele is reported. With pooling, a test for fit to Hardy-Weinberg is possible, and no significant deviation from Hardy-Weinberg was observed.

Table 2
Average per locus and mean heterozygosity over all loci for spiny lobster *Panulirus marginatus* from Maro Reef and Necker Island.

Location and date	*Est3*	*EstD*	*Gpi*	*Mpi*[1]	*Pgm*	*Tpep1*	*Tpep2*	Mean[2]
Maro Reef								
1987	0.061	0.000	0.059	0.381	0.045	0.356	0.049	0.021
Necker Island								
1987	0.015	0.000	0.087	0.477	0.030	0.389	0.039	0.023
Hawaiian Archipelago								
1978–80[3]	0.059	0.032	0.076	0.374	0.042	0.370	0.026	0.021

[1]Sex restricted allele distribution (Shaklee 1983).
[2]1987 estimate assumes monomorphism at the 39 additional loci surveyed by Shaklee and Samollow (1984).
[3]From Shaklee and Samollow (1984).

Chi-square analyses

Contingency chi-square analyses were performed between the two newly sampled collections at each of the polymorphic loci. Because of their low frequencies, all variant alleles at each locus were pooled into one class. Significant heterogeneity between Necker Island and Maro Reef was observed for *Est3* ($\chi^2 = 5.61$, df = 1, $P<0.020$).

Contingency chi-square analyses were also performed between the data gathered in this study and those of Shaklee and Samollow (1984) at each of the polymorphic loci. Significant heterogeneity was observed between the Necker Island 1987 and 1979–80 collections at *Est3* ($\chi^2 = 5.86$, df = 1, $P<0.025$).

Shaklee and Samollow (1984) reported fluctuating frequencies over years at the *EstD* locus and concluded that the 1980 samples represented a cohort with an unusual frequency. Therefore, we tested for heterogeneity between our data and the 1979 and 1980 *EstD* data separately. Both Maro Reef and Necker Island were significantly different for the 1980 comparison (Maro Reef, $\chi^2 = 9.71$, df = 1, $P<0.005$; Necker Island, $\chi^2 = 15.42$, df = 1, $P<0.001$). The Necker Island 1987 data were significantly different from the 1979 data ($\chi^2 = 6.10$, df = 1, $P<0.02$); the Maro Reef data were not significantly different.

Number of allelic classes

The total number of observed alleles at all sampled loci can also be used as an indicator of overall variability. Shaklee and Samollow (1984) observed a total of 18 different allelic classes in both the Maro Reef and Necker Island collections; we observed, for the same loci, 16 and 15 classes, respectively (Table 1). Although three classes (with frequencies of 0.010 or less) detected earlier were not observed in this study, two new alleles were observed.

It is also important to note the persistence of low-frequency allelic classes. *Gpi(s)*, *Pgm(f)*, and *Pgm(s)* were present in the 1978–80 Maro Reef collections at frequencies of 0.009 or less; all were present in the 1987 collection. *Gpi(s)* and *Mpi(f)* were present in the 1978–80 Necker Island collection at frequencies of 0.007 or less; both were present in the 1987 collection.

Heterozygosity

Heterozygosities were calculated for each locus for each population (Table 2). The *Est3* heterozygosities in the 1987 Necker Island collection were lower than those observed in the Hawaiian Archipelago during 1978–80 as no variants at this locus were observed. However, the 1987 Necker Island average heterozygosity over all loci was 0.023 (assuming monomorphism for the 39 additional loci previously resolved) compared with the value of 0.021 reported in the earlier study. Mean heterozygosities were statistically indistinguishable between the two populations and those observed in the earlier study. It should be noted, though, that differences on the order of 5% heterozygosities may require that large numbers of loci be screened to statistically test significant differences, and tests of heterozygosities are particularly insensitive when comparing low levels of heterozygosity such as observed in this study (Archie 1985).

Discussion

Stability of allele frequencies over at least 7 years was found at *Gpi*, *Mpi*, *Pgm*, *Tpep1*, and *Tpep2*. The gene frequencies of spiny lobster from Maro Reef and Necker Island appear to be stable both between collection sites and between years at all loci, with the exception of the two esterase loci. The variability at

the two esterase loci is of particular concern. Is this variability indeed indicative of genetic isolation between the two sampling localities or of changes in the stock structure?

The data of Shaklee and Samollow (1984) showed that *EstD* allele frequencies fluctuate in spiny lobster. They detected annual significant fluctuations in allele frequency within localities, but these annual fluctuations were parallel among Maro Reef, Necker Island, and an additional locality, Kure Atoll. Thus, the fluctuating frequencies did not lead to any significant differences in allele frequency between localities within years.

Our data, 7 years later, are somewhat consistent with this pattern for *EstD*. We detected significant differences between years, but no significant differences were found between localities sampled the same year. These data support the contention that fluctuations in the northwestern Hawaiian Islands do occur in *EstD* allelic frequencies. The data are insufficient to warrant invoking selection or drift as an explanation; however, one suitable hypothesis might be that these fluctuations were caused by immigration of a closely related population differing only at *EstD* frequencies.

However, unlike the previous data set, our data show a significant difference between the 1987 Necker Island and Maro Reef collections at *Est3*. Such change at a single locus could indicate either selection or stochastic processes operating on the *Est3* allelic frequencies with a lack of gene flow between the two localities.

No significant differences in levels of heterozygosity were detectable. This finding is not surprising. The rate of change in heterozygosity based on random drift is inversely proportional to population size (Hartl and Clark 1989) and is calculated as

$$H_t = (1 - 1/2\,N)^t\, H_0$$

where H_0 is the original heterozygosity value, H_t is the heterozygosity value after t generations, and N is the population size. At most, four generations have passed since the previous sampling. Thus, heterozygosity values should be indistinguishable with population sizes characteristic of a commercially exploitable species.

An analysis of low-frequency or rare alleles is often a more sensitive test to detect changes in variability. We observed a number of low-frequency alleles that were previously detected by Shaklee and Samollow (1984). Gregorius (1980) estimates that a sample size of 754 or greater would be needed to assure a 95% probability of detecting all alleles at a locus with frequencies of 0.01 or greater. Sample sizes were large in the earlier study—ranging from 386 to 666—increasing the probability of detection of rare alleles. Thus, it was expected that some of the previously detected rare alleles would not be observed in the 1987 collections, where sample sizes were 200 or less. Our data suggest that there has been no overall reduction in number of rare alleles within the populations; in fact, we detected rare alleles that were previously undetected.

The unchanged average heterozygosities and the persistence of rare alleles indicate that there has been no measurable loss of genetic variability due to fishing pressure. An additional concern in the management of the lobster fishery is whether each bank is primarily a self-recruiting population with minimal larval recruitment coming from other banks, or whether larval recruitment at any bank comes from an archipelago-wide gyre so the depletion of the spawning stock at any one bank will not affect recruitment to that bank. The significant difference between the 1987 Necker Island and Maro Reef frequencies at *Est3* is consistent with the hypothesis that the banks are largely self-recruiting, although this locus also appears to have varied significantly over time. Additional genetic studies, possibly including more sensitive DNA markers, are warranted to further elucidate the amount of genetic differentiation between these two important lobster populations. At present, though, a conservative management strategy would be based on the existence of two self-recruiting populations rather than a strategy based on a single panmictic unit.

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Citations

Allendorf, F., N. Ryman, and F. Utter
1987 Genetics and fishery management: Past, present, and future. *In* Ryman, N., and F. Utter (eds.), Population genetics and fishery management, p. 1–19. Wash. Sea Grant Prog., Univ. Wash. Press, Seattle.

Archie, J.W.
1985 Statistical analysis of heterozygosity data: Independent sample comparisons. Evolution 43:678–683.

Clayton, J.W., and D.N. Tretiak
1972 Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169–1172.

Gregorius, H-R.
1980 The probability of losing an allele when diploid genotypes are sampled. Biometrics 36:643–652.

Hartl, D.L., and A.G. Clark
1989 Principles of population genetics, 2d ed. Sinauer Assoc., Inc., Sunderland, MA.

May, B., J.E. Wright, and M. Stoneking
1979 Joint segregation of biochemical loci in Salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36: 1114–1128.

Nelson, K., and M. Soulé
1987 Genetical conservation of exploited fishes. *In* Ryman, N., and F. Utter (eds.), Population genetics and fishery management, p. 345–368. Wash. Sea Grant Prog., Univ. Wash. Press, Seattle.

Polovina, J.J.
1989 Density dependence in spiny lobster, *Panulirus marginatus*, in the Northwestern Hawaiian Islands. Can. J. Fish. Aquat. Sci. 46:660–665.

Shaklee, J.B.
1983 Mannosephosphate isomerase in the Hawaiian spiny lobster *Panulirus marginatus*: A polymorphic, sex-linked locus useful in investigating embryonic and larval sex ratios. Mar. Biol. (Berl.) 73:193–201.

Shaklee, J.B., and P.B. Samollow
1984 Genetic variation and population structure in a spiny lobster, *Panulirus marginatus*, in the Hawaiian archipelago. Fish. Bull., U.S. 82:693–702.

Swofford, D.L., and R.G. Selander
1981 BIOSYS-1—a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.