POPULATION STRUCTURE AND GENE FLOW IN BERMUDA's REEF FISH

ANNE F. GLASSPOOL
Bermuda Biological Station
17, Biological Station Lane
Ferry Reach
Bermuda GE 01

ABSTRACT

The source of recruits to populations of three reef fish species found in Bermuda, *Haemulon flavolineatum*, *Abudefduf saxatilis* and *Thalassoma bifasciatum* were examined through the technique of allozyme electrophoresis. Levels of genetic variation observed were within the range recorded for other marine teleosts. Allelic frequencies were compared between populations sampled from Bermuda, Miami, Bahamas and Barbados; no evidence of significant heterogeneity was observed between these populations of *Abudefduf saxatilis* and *Thalassoma bifasciatum*; however indications of population substructuring were observed in *Haemulon flavolineatum*, suggesting that gene flow was restricted and that genetic divergence in the Bermuda population had occurred. Reports in the literature indicate that this species has a relatively short larval residence period of two weeks, and it is proposed that this may not be sufficiently long to allow 'foreign' recruits to migrate to Bermuda. It may be inferred from this that the population of *H. flavolineatum* in Bermuda is largely self-replenishing, and if the limiting factor is the pelagic larval duration (PLD), then other species with similarly short PLD's might be expected to show evidence of stock heterogeneity. This study confirms the need for stringent local fisheries management policies, and adds further support for the implementation of a local fish pot ban in 1990.

INTRODUCTION

Despite their diversity, coral reef fish exhibit a remarkable uniformity in one aspect of their life history. With only one known exception (*Acanthochromis polyacanthus*), reef fish produce pelagic larvae, which spend variable periods of time (from two weeks to several months (Thresher, 1984)) in the water column prior to metamorphosing and entering the second phase of the life history, a fairly sedentary, site-attached existence among shallow reefs (Sale, 1978).

The presence of coral reef fish around oceanic islands isolated by hundreds of miles from any possible source of propagules, suggests that the larval stages may be capable of significant, long-distance dispersal. Indeed, observations on the spawning behaviour of many fish has led to the suggestion that reef fish employ

tactics which actually encourage such dispersal either to avoid predation by reef-dwelling planktivorous fish (Johannes, 1978), or to transport larvae to favourable feeding areas (Kiorboe et al., 1985), or as an adaptation to the instability of most reef systems, allowing colonisation of more favourable habitats and incidentally maintaining gene flow (Crisp, 1978; Barlow, 1981; Doherty et al., 1985).

However, offsetting the advantages of long range dispersal, is the higher mortality associated with increasing planktonic residence time, and hence loss of potential recruits. Indeed evidence has been presented which suggests that dispersal is responsible for enormous losses of fish larvae (Nelson et al., 1977; Sinclair et al., 1985; Peterman and Bradford, 1987; Walsh, 1987). Traditionally, it has been assumed that the supply of reef fish larvae competent to settle is in excess of the resources available to those larvae after settlement, however recent studies have revealed a number of recruitment-limited assemblages in which there are insufficient larvae settling from the plankton to saturate the available resources (Williams, 1980; Doherty, 1981 and Victor, 1983; Milicich et al., 1992). This presents a particularly significant problem for isolated fish populations.

The production of pelagic larvae would thus appear to serve two opposing demands, ensuring that sufficient numbers of larvae are retained within their natal reef to maintain the local population, whilst allowing some to escape to ensure gene flow. The extent of dispersal will be the product of the spawning behaviour of adults, hydrography, reef topography, behavioural characteristics of the larvae (in particular swimming behaviour on a vertical and horizontal scale), larval duration and predation. It will influence not only fish abundance on a local scale, but also large-scale distribution patterns and the genetic structure of natural populations.

In recent years, techniques of population genetics have been widely applied for the identification of geographically isolated fish stocks (Allendorf and Utter, 1979). Conspecific populations which are genetically isolated from each other will tend to evolve along different paths through the processes of genetic drift and natural selection, until eventually they can no longer successfully interbreed (Wright, 1931). Incomplete isolation may result in differentiation between populations within a species. The formulation of effective management strategies will depend on the extent to which a reef population is self-replenishing, or is dependent on recruits from 'upstream' fisheries. For island fisheries, conditions which lead to the dispersal of larvae away from the island and the recruitment of larvae from 'upstream' sources, will tend to result in high gene flow between populations. Over time, this will cause low speciation rates and widespread and genetically homogenous species distributions (Scheltema, 1978). At the other extreme, retention of larvae around oceanic islands, with subsequent local

recruitment, will lead to low gene flow, and the potential for genetic differentiation and subsequent endemism (Farmer and Berg, 1989).

The isolated island chain of Bermuda presents an ideal study model. Located in the central gyre of the western Atlantic Ocean, Bermuda comprises the world's northernmost coral reef system. It is biotically linked with the Caribbean, with a rich fauna and flora, although it does not exhibit the same level of species diversity; several reef fish common in the more tropical waters to the south, are absent locally. Their absence may be due to unsuitable environmental conditions prohibiting successful inhabitation, or it may be the result of BermudaÕs geographic isolation; the larvae of certain species may be incapable of undertaking the necessary migration. For those species which are found locally, the question remains as to whether their populations continue to be replenished by recruits from upstream sources, or whether they have become genetically isolated.

MATERIALS AND METHODS

Collection of specimens

Population samples of three species of reef fish, *Haemulon flavolineatum, Abudefduf saxatilis* and *Thalassoma bifasciatum* were collected from the Florida Keys, New Providence Bahamas and the west coast of Barbados, to be compared with samples from Bermuda. Collecting techniques varied; traps were employed where possible to collect samples of *Haemulon flavolineatum* but these were also supplemented by the use of a hook and line; similarly, line fishing was successful in some locations for Abudefduf saxatilis, but in Bermuda and Barbados trapping the schooling fish at the surface within a barrier net proved more effective; SCUBA divers using aquarium dip nets were most successful in collecting all the *Thalassoma bifasciatum* specimens. Where possible 50 individuals of each species were collected from each site. In Barbados, collection of *Haemulon flavolineatum* proved difficult, and only 44 specimens were sampled. In Miami, only 4 *Abudefduf saxatilis* individuals were collected, which was insufficient for electrophoretic analysis.

Screening Studies

Twenty two enzyme systems were screened to determine firstly the enzyme/buffer/tissue combination giving the best activity and resolution, and secondly, those systems exhibiting protein polymorphism. The enzyme systems selected for the main study were; Aspartate aminotransferase (AAT), Aconitate hydratase (AH), Creatine kinase (CK), Fumarate hydratase (FUM), Glucose-6-phosphate isomerase (GPI), Isocitrate dehydrogenase (ICDH), Lactate

dehydrogenase (LDH), Malate dehydrogenase (MDH), Malic enzyme (ME), Phosphoglucomutase (PGM) and 6-phosphoglucose dehydrogenase (6-PGD).

Tissue preparation and electrophoresis

All fish were kept in aerated water until they were killed, at which time their standard length was measured and their sex determined. The eyeball, a section of skeletal muscle and the liver were removed, placed separately in airtight PVC vials and snap frozen in dry ice. The samples were transported on dry ice back to Bermuda for electrophoretic analysis. The tissues were homogenised in an equal volume of deionised water and centrifuged at approximately 5,000 G in a sero-fuge for 5 minutes at room temperature. Whatman #4 paper wicks were soaked in the supernatant for 15 min at 4 oC and then inserted into horizontal starch gels (11.3% Sigma Starch) which had been prepared in a microwave. The gel buffer used was 0.005 M Tris-HCl/0.02 M MgCl2 (pH 7.5). Resolution of 11 enzyme systems was achieved using two different buffer systems described in Table 1. The gels were run for 14-17 hours at 4oC in a refrigerator.

Enzyme activity was detected by staining of the gels following the procedures of Shaw and Prasad (1970). All staining solutions were incorporated into a 1% agar overlay. The stained gels were incubated at 37oC until the banding patterns were clear enough to be scored. Presumptive gene loci for proteins with a single isozyme expressed were designated by the abbreviation for that enzyme. For proteins encoded by multiple gene loci, the loci were numbered according to decreasing anodal mobility. Allele products were numbered by migratory distance relative to the most common band which was numbered 100.

Data interpretation and analysis

Two methods were employed to determine that the observed banding patterns were genetic in origin. Firstly, the patterns were compared with those expected from other studies in which the genetic basis of the electrophoretic variation is known; this includes information on the subunit structure of each enzyme, the expected number of loci, the relative band strengths and the tissue specificity of isozyme expression in fishes. Secondly, the numbers of each phenotype (and hence presumed genotype) observed in each population sample were compared with those expected in a randomly mating population under conditions of Hardy-Weinberg equilibrium. Significant departures from this equilibrium (which were tested using the Chi-square statistic) were suggestive of banding patterns arising from non-genetic factors.

Allelic frequencies calculated from the electrophoretic data were analysed to provide estimates of average heterozygosity (H, Nei, 1978) and Wright's

standardised variance in allele frequencies (FST). FST was also used to determine Nm, which can be defined as the absolute number of migrants to each subpopulation, each generation. Contingency Chi-square tests were also performed to determine heterogeneity between populations sampled from different locations by testing for significant differences in allele frequencies. All analyses were conducted using BIOSYS-1 release 1.7 (Swofford and Selander, 1981).

RESULTS

Haemulon flavolineatum

10 enzyme systems were successfully resolved by gel electrophoresis and 18 presumptive gene loci were scored. Activity levels were generally high and resolution was typically well defined for these loci. Within the Bermuda sample, two loci (Icdh-1 and Pgm-1) showed variant allozymes (two and three alleles respectively). Chi-square contingency tests revealed no significant departures from genotypic frequencies expected under Hardy-Weinberg conditions ($P>0.05$).

The genetic structure of *H. flavolineatum* sampled from all four locations revealed certain variations. Average heterozygosity (H) ranged between 0.001 to 0.016, with the greatest genetic variability observed in the Bermudian and Barbadian populations. No variation was observed in the Icdh-1 locus outside Bermuda, but all sites were polymorphic for Pgm-1 and samples from the Bahamas and Barbados also showed variation at the Ck-2 locus. Only the latter population was polymorphic at the 0.95 level. With one exception, each population was within the Hardy-Weinberg equilibrium at each locus. The exception was observed at the Pgm-1 locus in the Barbados samples where there was a deficiency of heterozygotes, but this was less than one per homozygote class. Contingency Chi-square tests revealed significant differences for the Ck-2 ($X2\ [6] = 13.499$, $P<0.05$) and Icdh-1 ($X2\ [3] = 22.324$, $P<0.001$) loci between the four populations. Further analysis between pairs of populations revealed that the Bermudian population differed significantly at the Icdh-1 locus when compared with each of the other populations, which was not surprising given that the slower migrating allele was only observed in the Bermuda sample.

Values of Fst were calculated for each locus, and a mean value was also determined. The overall mean Fst value between all populations was 0.034 with a range of 0.024 for Pgm-1 and 0.058 for Icdh-1. The absolute number of migrants between each subpopulation, per generation was calculated from the Fst values as Nm: The overall mean was found to be low, 7.10 with a range of 4.06 (Icdh-1) to 10.17 (Pgm-1).

Abudefduf saxatilis

10 enzyme systems and 19 presumptive gene loci were resolved Within the Bermuda samples, 5 of the loci exhibited polymorphism (Aat-1, Fum-1, Gpi-1, Pgm-1 and 6Pgd1; of these, all produced two alleles except 6Pgd-1 for which it was concluded that three alleles were present. Only Aat-1, Fum-1 and 6Pgd-1 were polymorphic at the 0.95 level. The banding patterns expected of heterozygotes given the tertiary structure of these enzymes were not always clear, particularly for Aat-1, Fum-1 and 6Pgd-1. Contingency Chi-square tests to determine if there was any significant departure from Hardy-Weinberg equilibrium revealed significant results for the 6Pgd-1 locus (P=<0.05) indicating that there was a deficiency of heterozygotes. Caution was therefore exercised when considering this locus in subsequent analyses.

Samples from Bermuda were compared with those from the Bahamas and Barbados and were not found to be significantly different at any loci. 6Pgd-1 was found to be deficient in heterozygotes at the first two locations so calculations of FST were performed both with and without this locus. A mean Fst value of 0.006 was calculated (range 0.001 to 0.009) between loci. A mean migration rate (Nm) of 41.42 was calculated with a range between 27.53 for Aat-1 and Fum-1 and 249.75 for Pgm-1. Average heterozygosity (H) was found to be slightly higher in the Bermuda samples (0.087 compared to 0.085 in the Bahamas and 0.081 in Barbados).

Thalassoma bifasciatum

Ten enzyme systems were found to show strong activity and excellent resolution, and these yielded 17 presumptive gene loci. Variation was observed in 7 of these with two alleles scored except where indicated; Ah-1 (4 alleles), Gpi-1, Gpi-2, Idh-1 (5 alleles), Ldh-1, Mdh-1 and Pgm-1 (3 alleles). For each locus the banding pattern observed for heterozygous individuals conformed to that expected from the known tertiary structure. As a further test, contingency Chi-square tests were performed. The Chi-square result was significant for the Ldh-1 locus but there was only one variant allele in the whole sample so this result is probably attributable to sampling error.

With one exception, samples collected from the Caribbean locations all conformed to the Hardy-Weinberg equilibrium; the exception was the Ah-1 locus in the Barbados sample in which there was an excess of homozygotes. Contingency Chi-square tests revealed no significant differences between the Ah-1, Gpi-2, Idh-1, Ldh-1 and Mdh-1 loci; a significant result was obtained at the Pgm-1 locus (P<0.05>0.01). Migration rates (Nm) were calculated from Fst values and ranged between 13.64 for Pgm-1 and 31.0 for Gpi-2, Ldh-1 and Mdh-1, with a mean value

of 20.58. Greatest genetic variability was observed in the Miami sample (H = 0.076) and least in Bermuda (H = 0.061).

DISCUSSION

The technique of gel electrophoresis was sucessfully employed to compare the allelic frequencies of three species (*Haemulon flavolineatum, Abudefduf saxatilis* and *Thalassoma bifasciatum*) from geographically isolated populations. Genetic variability was observed in all three species, and the banding patterns observed for most of the enzyme systems exhibiting polymorphic loci conformed to expectations from previous studies of gene expression and subunit compositon. The exceptions were FUM and AAT in *Abudefduf saxatilis* for which the heterozygote typically appeared as a smeared region between the fast and slow allozyme migration points. However close agreement between the observed frequencies of presumed genotypes and those expected on the basis of Hardy-Weinberg equilibrium supports the presumption that the variation observed was genetic in origin for these loci. The 6Pgd-1 locus exhibited an apparent excess of homozygotes, and caution was therefore exercised with this result.

All three species exhibited levels of genetic variation comparable to those reported for other fish species, with a range of H= 0.008 for *Haemulon flavolineatum* to H= 0.084 for *Abudefduf saxatilis* giving a mean value of H = 0.044. Smith and Fujio (1982) calculated a mean value of H = 0.06 +/- 0.038 (with a range of H = 0 - 0.146), for 89 species of marine teleosts. Waples (1986) reported a mean heterozygosity value of H = 0.031 for 10 species of marine shore fishes, with a highest value of H = 0.087; whilst Shaklee (1984) determined that the variation in the Pacific damselfish *Stegastes fasciolatus* was H = 0.046.

Stock homogeneity between Bermuda's reef fish and those in the Caribbean was examined and the results indicate that levels of genetic differentiation between widely separated populations of *Abudefduf saxatilis* and *Thalassoma bifasciatum* were insignificant. The lack of population subdivision suggested by the Chi-square analyses is supported by the low FST values (FST = 0.006 and 0.012 respectively). However, for *Haemulon flavolineatum* significant Chi-square results for two of the three polymorphic loci analysed indicate that the population is not panmictic; rather the Bermuda population appears to be differentiated from Caribbean populations. An FST value of 0.034 was concordant with this result. The calculated Nm value of 7.1 is lower than for many other marine shore fishes (Waples, 1986) suggesting that the rate of exchange of migrants between populations, is not as frequent as in many other species.

Studies in the Indo-Pacific have revealed several species which show significant genetic differentiation among localities (eg. Bell et al., 1982; Waples,

1986). However, in the Western Atlantic no electrophoretic studies on reef fish species have been undertaken that have included Bermuda's populations (with the exception of Hateley, in prep.) for comparison. A study by Mitton et al. (1989) investigated the population structure of the Queen Conch, *Strombus gigas*. They reported significant variation in the allele frequencies of this species in Bermuda, prompting them to conclude that the local population was genetically isolated from the Caribbean. They reported a value of Nm = 8.7 which is similar to the Nm value calculated in the present study.

It has long been assumed that the Gulf Stream provides a transport mechanism for the larval stages of the shallow water benthic species found in Bermuda (Tee-Van, 1930; Beebe and Tee-Van, 1933; Collette, 1962). Whilst difficult to prove directly, Scheltema (1968) provided evidence in support of this. He found veliger larvae of the tropical gastropod *Cymatium parthenopeum* distributed throughout the track of the Gulf Stream from Florida to the Azores. Further evidence of its role as a transport vector comes from ichthyoplankton studies; frequently the larvae of tropical and subtropical families are sampled in waters outside the range of the adults (Markle et al., 1980; Evseenko, 1982; Powell and Robbins, 1994).

In order for larvae spawned in the Caribbean to reach Bermuda, they must be entrained in an eddy or ring which separates from the main flow of the Gulf Stream and impinges on Bermuda. Hogg (1972) found evidence of such rings, as did Spitzer (1985), who proposed that mesoscale anticyclonic eddies may arrive in the vicinity of Bermuda with an average frequency of 2-6 times per year. A recent study by Schultz and Cowen (1994) used satellite imagery to examine Gulf Stream trajectories. They applied various models and concluded that the larval duration of most reef fish species was insufficiently long to allow for frequent transport from the Caribbean. Further they proposed that the recurrence of hydrographic events which do bring water rapidly enough to carry viable larvae, would exceed the lifespan of any species with a pelagic larval duration exceeding a month.

The absence of significant genetic differentiation between populations of *Abudefduf saxatilis* and *Thalassoma bifasciatum* suggests that the larvae of both these species can remain planktonic for sufficiently long periods to allow their transport to Bermuda. Otolith examination of these species has indicated that the pelagic larval durations of these species are 3-4 weeks for *A. saxatilis* (Thresher, 1984) and 6-10 weeks for *Thalassoma bifasciatum* (Victor, 1982; Brothers and Thresher, 1985). In contrast *Haemulon flavolineatum* has a planktonic larval duration of just two weeks (Thresher, 1984), and it is proposed that this may impose a limit on the dispersal ability of this species. The average larval development period of *Strombus gigas* mentioned above, is 21 days. In the absence of a large-

scale genetic analysis of larval *H. flavolineatum* however, the possibility that the limiting factor may be the presence of unfavourable local conditions prohibiting settlement rather than a short larval duration, cannot be excluded. Nevertheless, the results suggest that populations of *H. flavolineatum* in Bermuda must be largely self-seeding and management policies must take this into consideration. Further work is required to determine if species with similar larval residence periods also show evidence of genetic differentiation in Bermuda. In this light it is interesting to note that of 14 species of Haemulids found in the Western Atlantic, only 7 species have been recorded from Bermuda.

ACKNOWLEDGEMENTS

I am indebted to the staff at the Bermuda Government Division of Fisheries under the direction of John Barnes, and the Bermuda Aquarium, Museum and Zoo for their logistic support throughout this research program. In particular I would like to thank Jack Ward, Brian Luckhurst, Billy Mitchell, Lance Furbert and the late Frank Ray. My thanks also go to Jon Hateley for his advice regarding the gel electrophoresis. In the collection of the specimens, I was assisted by many people: Chris Jones and Clay Porch from National Marine Fisheries Service and Jeff Silberman at the Rosenstiel School of Marine and Atmospheric Science, University of Miami; Gary Miller and his staff at the Coral World Aquarium in New Providence, Bahamas; and Barry Bjork for his help in Barbados Financial assistance was provided by The Royal Society.

LITERATURE CITED

Allendorf, F. W. and F. M. Utter. (1979). Population Genetics. In: Fish Physiology, 8 (eds. W. S. Hoar, D. J. Randall and J. R. Brett). Academic Press, New York, 407-454.

Barlow, G. W. (1981). Patterns of parental investment, dispersal and size among coral reef fishes. Environmental Biology of Fishes, 6, 65-85.

Bell, L. J., J. T. Moyer & K. Numachi (1982). Morphological and genetic variation in Japanese populations of the anemonefish Amphiprion clarkii. Marine Biology, 72, 99-108.

Brothers, E. B. & R. E. Thresher (1985). Pelagic duration, dispersal and the distribution of Indo-Pacific coral reef fishes. In: The Ecology of Coral Reefs. (ed. M. L. Reaka), N.O.A.A. Symposia Series for Undersea Research, 3, 53-69.

Crisp, D. J. (1978). Genetic consequences of different reproductive strategies in marine invertebrates. In: Marine Organisms: genetics, ecology and evolution. Plenum Press, New York, 257-273.

Doherty, P. J. (1981). Coral Reef Fishes: Recruitment - limited assemblages. Proceedings of the 4th International Coral Reef Symposium, Manila, 2, 465-470.

Doherty, P. J., D. M. B. Williams & P. F. Sale (1985). The adaptive significance of larval dispersal in coral reef fishes. Environmental Biology of Fishes, 12, 81-90.

Evseenko, S. A. (1982). Ichthyoplankton of Slope and Gulf Stream Waters off Nova Scotia in Late Autumn 1974. Journal of the Northwest Atlantic Fishery Science, 3,127-139.

Farmer, M. W. & C. J. Berg (1989). Circulation around islands, gene flow, and fisheries management. Proceedings of the Gulf and Caribbean Fisheries Institute, 39, 318-330.

Hogg, N. G. (1972). Steady flow past an island with applications to Bermuda. Geophysics and Fluid Dynamics, 4, 55-81.

Johannes, R. E. (1978). Reproductive strategies of coastal marine fishes in the tropics. Environmental Biology of Fishes, 3, 65-84.

Kiorboe, T., P. Munk & J. G. Stottrup (1985). First feeding by larval herring Clupea harengus L. Dana, 5, 95-107.

Markle, D. F., W. B. Scott & A. C. Kohler (1980). New and Rare Records of Canadian Fishes and the Influence of Hydrography on Resident and Nonresident Scotian Shelf Ichthyofauna. Canadian Journal of Fisheries and Aquatic Sciences, 37, 49-66.

Milicich, M. J., M. G. Meekan, & P.J. Doherty (1992). Larval supply: a good predictor of recruitment of three species of reef fish (Pomacentridae). Marine Ecology Progress Series, 86, 153-166.

Mitton, J.B., C.J. Berg & K.S.Orr(1989). Population structure, larval dispersal, and gene flow in the queen conch,Strombus gigas, of the Caribbean. Biological Bulletin, 177, 356-362.

Nei, M. (1978). Estimation of the average heterozygosity and genetic distance from a small number of individuals. Genetics, 89, 583-590.

Nelson, W. R., M. C. Ingham & W. E. Schaef (1977). Larval transport and year class strength of Atlantic Menhaden, Brevoortia tyrannus. Fishery Bulletin, 75, 23-41.

Peterman, R. M. & M. J. Bradford (1987). Wind speed and mortality rate of a marine fish, the Northern Anchovy (Engraulus mordax). Science, 235, 354-355.

Powell, A. B. & R. E. Robbins (1994). Abundance and distribution of icthyoplankton along an inshore - offshore transect in Onslow Bay,

North Carolina. NOAA Technical Report NMFS, U.S. Department of Commerce, 120, 1-28.

Sale, P. F. (1978). Coexistence of coral reef fishes - a lottery for living space. Environmental Biology Of Fishes, 3, 85-102.

Scheltema, R. S. (1978). On the relationship between dispersal of pelagic larvae and the evolution of marine prosobranch gastropods. In: Marine Organisms, Genetics, Ecology and Evolution. (eds. B. Battaglia and J. Beardmore). Plenum, New York, 391-397.

Schultz, E. T. & R. K. Cowen. (1994). Recruitment of coral-reef fishes to Bermuda: local retention or long - distance transport? Marine Ecology Progress Series, 109, 15-28.

Shaklee, J. B. (1984). Genetic variation and population structure in the damselfish, Stegastes fasciolatus, throughout the Hawaiian Archipelago. Copeia, 629-640.

Shaw, C. R. & R. Prasad. (1970). Starch gel electrophoresis of enzymes - a compilation of recipes. Biochemistry and Genetics, 4, 297-320.

Sinclair, M., M. J. Tremblay & P. Bernal (1985). El nino events and variability in a Pacific mackerel (Scomber japonicus) survival index:support for HjortÕs second hypothesis. Canadian Journal of Fisheries and Aquatic Science, 42, 602-608.

Smith, P. J., & Y. Fujio (1982). Genetic variation in marine teleosts: high variability in habitat specialists and low variability in habitat generalists. Marine Biology, 69, 7-20.

Spitzer, W. S. (1989). Rates of vertical mixing, gas exchange, and new production: estimates from seasonal gas cycles in the upper ocean near Bermuda. Ph.D. Thesis, Woods Hole Oceanographic Institute 89-30, 122 pp.

Swofford, D. L. & R. B. Selander. (1981). BIOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity, 72, 281-283.

Thresher, R. E. (1984). Reproduction In Reef Fishes. T. F. H. Publications, New Jersey.

Victor, B. C. (1982). Daily growth increments and recruitment in two coral reef wrasses, Thalassoma bifasciatum and Haliochoeres bivittatus. Marine Biology, 71, 203-208.

Victor, B. C. (1983). Recruitment and population dynamics of a coral reef fish. Science, 219, 419-429.

Walsh, W. J. (1987). Patterns of recruitment and spawning in Hawaiian reef fishes. Environmental Biology of Fishes, 18, 257-276.

Waples, R. S. (1986). A multispecies approach to the analysis of gene flow in marine shore fishes. Ph.D. Dissertation University of California, San Diego.

Waples, R. S. & R. H. Rosenblatt. (1987). Patterns of larval drift in southern California marine shore fishes inferred from allozyme data. Fishery Bulletin, 85, 1-11.

Williams, D. M. (1980). Dynamics of the pomacentrid community on small patch reefs in One Tree Lagoon (Great Barrie Reef). Bulletin of Marine Science, 30, 159-170.

Wright, S. (1931). Evolution in Mendelian populations. Genetics, 6, 111-178.

TABLE 1. Number of presumptive gene loci scored for each enzyme and the buffers and tissues used for each species.Aa= Aspartate aminotransferase, Ah= Aconitate hydratase; Ck= Creatine kinase; Fh= Fumarate hydratase; G6= Glucose-6-phosphate isomerase; Id= Isocitrate dehydrogenase; Ld= Lactate dehydrogenase; Md= Malate dehydrogenase; Me= Malic enzyme; Pg= Phosphoglucomutase; 6Pg= 6-Phosphogluconate isomerase

Enzyme system	E.C. number	Buffer	# of loci/tissue H.flavolineatum	# of loci/tissue *A. saxatilis*	# of loci/tissue *T. bisfaciatum*
Aa	2.6.1.1.	M	2/L	2/L	2/L
Ah	1.1.1.1	C	***	1/L	1/L
Ck	2.7.3.2	C	3/E	1/E	1/E
Fh	4.2.1.2	M	1/L	1/M	1/M

G6	5.3.1.9	C	2/E	2/M	2/M
Id	1.1.1.42	C	2/L	1/L	1/L
Ld	1.1.1.27	C	3/E	3/E	3/E
Md	1.1.1.37	M	***	1/E	1/E
Me	1.1.1.40	M	1/L	2/M	2/M
Pg	2.7.5.1	C	1/M	1/M	1/M
6Pg	1.1.1.44	C	1/L	1/M	***

*** = enzyme system not resolved
M = 0.05M Tris-maleate pH 7.8; C = 0.155M Tris/0.043M Citric acid pH 7.0
E = Eye; L = Liver; M = Muscle

TABLE 2. Allelic frequencies of polymorphic loci for Bermuda and Caribbean samples of *Haemulon flavolineatum*. N = Number of individuals

LOCUS	ALLELE	BERMUDA	MIAMI	BAHAMAS	BARBADOS
Ck-2	N	50	50	50	44
	110	0.000	0.000	0.000	0.011
	100	1.000	1.000	0.990	0.943
	90	0.000	0.000	0.010	0.045
Idh-1	N	44	50	50	44
	100	0.924	1.000	1.000	1.000
	80	0.076	0.000	0.000	0.000
Pgm-1	N	44	50	50	44

	130	0.057	0.010	0.000	0.034
	100	0.920	0.990	1.000	0.943
	66	0.023	0.000	0.000	0.023

H	0.016	0.001	0.001	0.012
(S.E.)	0.011	0.001	0.001	0.012
P(0.95)	0.000	0.000	0.000	11.110
P(0.99)	11.110	5.560	5.560	11.110

TABLE 3. Allelic frequencies of polymorphic loci for Bermuda and Caribbean samples of *Abudefduf saxatilis.* N = Number of individuals.

LOCUS	ALLELE	BERMUDA	BAHAMAS	BARBADOS
Aat-1	N	40	50	50
	100	0.525	0.540	0.630
	70	0.475	0.460	0.370
Fum-1	N	48	50	50
	100	0.563	0.640	0.670
	60	0.438	0.360	0.330
Gpi-1	N	48	50	50
	100	0.969	0.980	0.990
	80	0.031	0.020	0.010
Pgm-1	N	50	50	50

	100	0.969	0.970	0.960
	75	0.031	0.030	0.040
6Pgd1	N	54	50	50
	140	0.056	0.070	0.040
	120	0.444	0.390	0.410
	100	0.500	0.540	0.550

H	0.087	0.085	0.081;
S.E.	0.044	0.043	0.041
P (0.95)	15.79	15.79	15.79
P (0.99)	26.32	26.32	26.32

TABLE 4. Allelic frequencies of polymorphic loci for Bermuda and Caribbean samples of *Thalassoma bisfaciatum*. N = Number of individuals

LOCUS	ALLELE	BERMUDA	MIAMI	BAHAMAS	BARBADOS
Ah-1	N	50	50	50	50
	110	0.080	0.050	0.040	0.130
	105	0.030	0.040	0.030	0.050
	100	0.880	0.880	0.900	0.790
	85	0.010	0.030	0.030	0.030
Gpi-2	N	50	50	50	50
	150	0.010	0.030	0.040	0.010
	100	0.990	0.970	0.960	0.990
Idh-1	N	50	50	50	46
	180	0.020	0.000	0.010	0.022

	150	0.170	0.120	0.140	0.098
	125	0.280	0.350	0.250	0.413
	100	0.480	0.450	0.540	0.402
	75	0.050	0.080	0.060	0.065
Ldh-1	N	50	50	50	50
	150	0.000	0.000	0.090	0.000
	100	1.000	1.000	0.910	1.000
Mdh-1	N	50	50	50	50
	225	0.040	0.100	0.090	0.100
	100	0.960	0.900	0.910	0.900
Pgm-1	N	50	50	50	50
	150	0.010	0.000	0.000	0.020
	100	0.970	0.910	0.960	0.970
	50	0.020	0.090	0.040	0.010

H	0.061	0.076	0.068	0.075
S.E	0.04	0.041	0.038	0.043
P(0.95)	11.76	23.53	17.65	17.65
P(0.99)	29.41	29.41	35.29	29.41