# ANALYSIS OF GENE FLOW OF THE BERMUDA SPINY LOBSTER

## O.D.A. PROJECT R4537

## FINAL REPORT

J.G. HATELEY (OCTOBER 1991)

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## CONTENTS

## SUMMARY

The source of recruits to Bermuda's spiny lobster (*Panulirus argus*) stock was examined through genetic typing techniques. A total of eight polymorphic loci were found in adults, sub-adults and juveniles, four in post-larvae and two in mid to late stage phyllosoma. Polymorphic loci were lost in these early life-history stages due to weak activity and ontogenetic changes at settlement. No enzyme activity was found in encapsulated larvae or early-stage phyllosoma.

No convincing evidence for population substructure was found within the Bermuda stock when genetically tested by spatial distribution, sex and size-class. Tests for macrogeographic heterogeneity using Menzies' (1981) data-base for Caribbean stocks were complicated by uncertainty regarding electromorph compatibility and temporal stability of allele frequencies. Genetic comparisons with contemporary samples obtained from south Florida demonstrated no significant heterogeneity. Monthly post-larval samples were in genetic equilibrium and indistinguishable from Bermuda and Florida adult stocks.

Three eddies approached to within 60 nautical miles of Bermuda and two attempts were made to strategically sample eddy 'B' waters for phyllosoma. The role of warm-water outbreaks and cold-core eddies in larval transport and recruitment to Bermuda, however, remains uncertain, as does the relative contributions of non-Bermuda and Bermuda derived larvae to the resident stock.

These results are consistent with a single Florida/western Atlantic stock. Increased co-operation with Florida State and United States Federal authorities is therefore recommended for effective resource management. However, as significant self-replenishment has not been excluded by this study, and in view of stable annual post-larval recruitment and the recent exploitation rates being sustainable, current fisheries regulations in Bermuda appear appropriate.

Preliminary results are also presented on species identification of *Panulirus* phyllosoma and comparisons in collecting efficiencies between three post-larval collector designs.

## INTRODUCTION

The successful management of a fishery requires an understanding of the biological principles underlying the resource. Information on the species distribution, abundance, population dynamics, reproduction and basic biology are all of value. However, knowledge of stock structure is of critical importance for sound management practices. In the absence of better information, stocks are generally considered to be self-replenishing and management policies are developed accordingly (Shaklee and Samollow, 1984).

Although these policies may be appropriate for relatively sedentary animals with short planktonic larval stages, they may not be suitable for species with protracted planktonic larval stages. These larvae are susceptible to considerable dispersion by oceanic currents and other hydrodynamic events, and may provide a mechanism for exchange between stocks and

subsequent gene flow. If larvae of 'foreign' origin were to recruit significantly to 'downstream' stocks, management policies based on self-replenishment at those sites would be inappropriate. Instead a regional management plan encompassing all interacting stocks would be required.

Spiny lobsters (*Palinuridae*) have prolonged planktonic larval stages, estimated as being 6-14 months for *Panulirus argus* (Lewis 1951; Farmer, Ward and Luckhurst, 1989; Sims and Ingle, 1967), 9-11 months for *P. cygnus* (Phillips, 1981) and approximately 8 months for *P. interruptus* (Johnson, 1960). The phyllosomal larvae pass through approximately eleven stages (Lewis, 1951) before metamorphosis to an actively swimming puerulus, or post-larva. Upon settlement, the commercially exploitable benthic stage is assumed.

*P. argus* constitutes one of the largest lobster fisheries in the world with annual landings typically averaging 64 million pounds (FAO statistics; in Williams, 1988). It is the staple fishery in many developing Caribbean countries, including Belize, Honduras and Nicaragua. Unfortunately, an incomplete understanding of the interrelationships of lobster populations with oceanic current systems has resulted in a threat of over-exploitation in many countries (Villegas, Jones and Labisky, 1982). There is therefore an essential need to establish the extent of larval exchange as a basis for regional co-operation in the development of policies to conserve these fisheries.

Several approaches have been used to address the problem of pelagic dispersal of organisms including analysis of oceanic currents, release and retrieval of marked drifters, tagging studies and electrophoretic analysis of protein polymorphisms and mitochondrial DNA variability. Sufficient data regarding Gulf Stream currents is available to construct models capable of predicting larval distribution over long distances. However, few reliable data exist regarding the inshore currents both at the sites of spawning and ultimate recruitment. Marked drifters can provide biologically relevant data regarding current patterns but these are usually restricted to surface currents and rely on the human population for retrieval, or on expensive remote sensing devices. Tagging studies are not well suited for the study of larval drift. For the above reasons, electrophoretic analysis has been used to study patterns of larval drift and the extent of gene flow in marine organisms.

A number of electrophoretic studies have been conducted on *Palinurid* populations. Shaklee and Samollow (1984) employed allozyme electrophoresis to investigate *P. marginatus* in the Hawaiian archipelago. The same techniques have been used to study *Jasus edwardsii/Jasus novaehollandiae* (Smith, McKoy and Machin, 1980) and *P. ornatus* (Salini *et al*, 1985) in Australasia. Both mtDNA analysis (McLean, Okubo and Tracey, 1983) and allozyme electrophoresis (Menzies, 1981; Menzies and Kerrigan, 1979), apparently demonstrated inter-population variation in *P. argus*. Menzies (1981) used eight polymorphic loci to examine six Caribbean populations, and demonstrated significant differences between most of them. However, the relative contributions of post-settlement selection and genetic drift to the differentiation were undetermined.

Menzies' research did not include an investigation of the Bermudian *P. argus* population, at the northern limit of its distribution. The spiny lobster constitutes an important fishery resource on the island. Net annual landings are in the region of 53,000 lbs, and make up a measurable part of the gross national product of the country (Villegas, Jones and Labisky, 1982). Increasing interest in the lobster fishery has developed over recent months as declining

reef-fish stocks necessitated an indefinite ban on traditional fish-traps (Bermuda Fisheries Management Plan, 1990).

The fishery is currently managed on the assumption that all new recruits originate from the local population. However, Bermuda is associated with hydrodynamic processes that promote both larval dispersal from the island and larval recruitment from 'upstream' sources (Farmer and Berg, 1989). Bermuda is situated within the North Atlantic gyre and is regularly approached by warm-water outbreaks and cold core eddies that spin off the Gulf Stream (Parker, 1971). Phyllosoma are known to be associated with Australian eddy waters (McWilliam and Phillips, 1983) and Atlantic eddies may also act as vectors for 'foreign' recruitment.

The aim of this project is to determine whether the Bermuda *P. argus* stock is self-replenishing, through the utilisation of genetic typing techniques.

i) Electrophoretic phenotypes will be defined for Bermuda adults and juveniles, and compared with Menzies' data-base for Caribbean sub-populations. Samples from contemporary Caribbean sub-populations will also be examined to investigate temporal stability of allele frequency.

ii) Larvae from nearshore and offshore waters and newly recruited post-larvae will be compared with the above samples to determine their genetic relatedness to Bermuda and Caribbean stocks.

iii) Larvae strategically sampled from eddies impinging on Bermuda will also be compared with newly recruited post-larvae and Bermuda adults.

Such tests would greatly enhance our knowledge of the interactions between lobster populations and hydrodynamic events (Farmer and Berg, 1989). They would also provide a basis for developing sound fisheries management policies in Bermuda and the Caribbean, perhaps strengthening support for a unified pan-Caribbean management programme. Ultimately, final resolution of the problem of stock retention would benefit all island coastal fisheries for a wide range of commercially important species.

## MATERIALS AND METHODS

### 1) SAMPLE COLLECTION

Approximate sampling areas are shown in Figure 1.

Specimens obtained from the Bermuda platform (<20 m depth) with carapace length greater than 50 mm were collected by Bermuda Aquarium and Fisheries personnel by trapping. Juveniles were obtained by free-diving, SCUBA and trapping. Colour morph (red/purple carapace) individuals from the platform edge were obtained from experimental lobster traps deployed by fishermen in 40-80 m water. The size and sex of each specimen were recorded and individuals were categorised by size and location of capture:

Platform legal ('platleg'), carapace length >92 mm.
Platform sub-legal ('platsub'), carapace length 50-92 mm.
Platform juvenile ('juvenile'), carapace length 25-50 mm.
Edge sub-legal ('edgesub'), carapace length 50-92 mm.

Specimens from Miami were obtained by local fishermen and forwarded by Chow (University of Miami). Florida Keys specimens were imported commercially into Bermuda, their origin was confirmed by two independent suppliers as the Florida Keys west of Marathon.

Post-larvae were obtained using Witham type habitats (Witham, Ingle and Joyce, 1968). A total of 16 collectors were deployed along the North shore at sites indicated as being good collecting areas by previous studies (Ward, 1989) (Figure 1). Three types of collector were employed; Nomad, DNR and Calinsky, each type incorporating different leaf materials and leaf numbers. Collectors were checked weekly and all post-larvae were removed. Animals were returned to the laboratory and kept in aerated sea-water prior to tissue preparation. Once 100 animals had been collected for a given month, additional animals were relocated in mangrove water distant from any collector station. Sampling commenced in January 1990 and continued until September 1991.

Phyllosoma larvae were collected at night by plankton tows. Tows were normally conducted at 2 knots for 20 minutes using a 500 $\mu$m plankton net with a 1m diameter circular opening. A General Oceanics flow-meter (model 2030) was positioned in the centre of the net and an average of 657 $m^3$ water was sampled at each station (s.d. = 37.3). Most tows were conducted just below the water surface but later some tows were down-rigged to approximately 10m depth. The plankton was washed in aerated sea-water for subsequent sorting and freezing at the laboratory. *Palinurid* larvae were coarsely staged into three size categories; small (approximately corresponding to stages I-IV; Lewis, 1951), mid (stages V-VIII) and large (stages IX-XI).

Gulf Stream warm-water outbreaks and eddy activity were followed through twice-weekly satellite maps of surface water temperatures obtained via facsimile from NOAA/National Ocean Service. Strategic sampling of Gulf Stream frontal waters was estimated through variations in surface water temperature, measured by thermometer.

## 2) SCREENING STUDIES

Screening studies were conducted at the Marine Science Laboratories, Menai Bridge and were continued in Bermuda. Effort concentrated on resolving the eight polymorphic loci utilised in earlier allozyme studies on *P. argus* (Menzies and Kerrigan, 1979; Menzies, 1981), with a number of additional systems considered promising in decapod crustaceans. The following enzyme systems were screened: acid phosphatase, ACP (umbelliferyl phosphatase, 'UP'; Menzies, 1981); esterase, EST; fumarase, FH; glutamate dehydrogenase, GLUD; glucose-6-phosphate isomerase, GPI; hexokinase, HK; isocitrate dehydrogenase, IDH ('ICD'; Menzies, 1981); lactate dehydrogenase, LDH; malate dehydrogenase, MDH; malic enzyme, ME; octanol dehydrogenase, ODH; peptidase-B, PEP-B; peptidase-D, PEP-D; phosphoglucomutase, PGM; sorbitol dehydrogenase, SDH; superoxide dismutase, SOD; 6-phosphoglucose dehydrogenase, 6PGDH; and xanthine dehydrogenase, XDH.

## 3) TISSUE PREPARATION AND ELECTROPHORESIS

Tissue preparation and electrophoresis was modified from Menzies (1981). Approximately 200-500 mg of tail muscle, leg muscle and hepatopancreas were dissected from adults and juveniles. Post-larvae were divided into cephalothorax, abdomen and legs; phyllosoma were sampled as whole body. All tissue samples were placed in 1.8 ml Eppendorf cryotubes, snap-frozen in liquid oxygen and stored at -20°C in a laboratory freezer.

An equal volume of deionised water was added to each sample and the tissue mechanically homogenised using a metal rod. Samples were centrifuged at approximately 5000G in a sero-fuge for 5 minutes at room temperature. Filter paper wicks (Whatman # 3) were soaked in the supernatent for 10 minutes at 4°C.

Most systems were run on horizontal starch gels (11.3% Sigma starch). Gels were prepared in a microwave with vigorous swirling every 20 seconds. Gel buffer was 0.005 M Tris - HCl/0.02 M $MgCl_2$ (pH 7.5). Electrode buffer was 0.05 M Tris/0.02 M maleate (pH 7.8) for MDH; 0.3 M borate (pH 8.6) for the remaining systems. Gels were run for approximately 18 hours at 4°C in a refrigerator. Esterases were analysed on horizontal 6.3% polyacrylamide gels; gel buffer was 0.37 M Tris - HCl/0.1% Triton X-100 (pH 8.6); electrode buffer 0.05 M Tris - HCl (pH 9.2). Gels were run for 1 hour at 4°C.

Enzyme activity was visualised according to standard staining procedures (Harris and Hopkinson, 1976; Shaw and Prasad, 1970). Substrates for PEP-B and PEP-D were leu-gly and phe-pro respectively. All staining solutions (with the exception of ACP) were incorporated in a 1% agar overlay.

For each locus, allele products were numbered according to their mobility relative to the most common allele (= *100*).

## 4) STATISTICS

All genetic analyses were conducted using BIOSYS-1 release 1.7 (Swofford and Selander, 1981). Conformity to Hardy-Weinberg expectations was tested by the chi-square goodness-of-fit test, with expected frequencies calculated using Levene's formula for small sample-sizes. Exact significance probabilities were also calculated as this test is superior for diallelic loci with low expected frequencies in some classes. Heterogeneity among sub-populations and samples was tested by the contingency chi-square statistic.

No calculations of genetic variability were performed. As this study was primarily concerned with between-stock and between-sample comparisons, effort concentrated on polymorphic loci and most monomorphic loci were abandoned. Consequently estimations of average heterozygosity would have been unacceptably biased.

One-way analysis of variance was conducted using the MINITAB release 7 statistical package.

## RESULTS

### 1) SCREENING STUDIES

The results of the screening experiments are summarised in Table 1.

A total of seven enzyme systems, consisting of nine polymorphic loci were initially selected for analysis; ACP, LDH, PGM, PEP-B, PEP-D, SOD, GPI. Later, ACP was excluded but two MDH loci (one weakly polymorphic) were incorporated in the scoring programme.

ACP appeared to exhibit two loci with the more anodal locus behaving as a three allele polymorphism. Scoring of this locus was eventually discontinued as increased frequencies of anodal bands were noted with freezer time, implying sample degradation was a possible source of the observed variation.

LDH displayed two loci; *LDH-2* from tail muscle was considered monomorphic in the samples studied (n = 271 individuals), *LDH-1* was weakly polymorphic and scored for two alleles (*100* and *110*) with the heterozygote generating the expected five-banded pattern. Specimens from the Florida Keys also displayed an additional slow band ('*30*'), however, this was not considered allelic in origin as the apparent heterozygotes displayed either two or three-band phenotypes. *LDH-1* was scored in all benthic stages but weak activity in larvae precluded scoring.

PGM activity was confirmed to a single locus with three co-dominant alleles (*100*, *200* and *-200*). This locus was also scored in post-larvae, juveniles and adults but not in phyllosoma.

Hepatopancreas PEP-B activity was scored as two polymorphic loci, both monomeric in structure. *PEP-B1* exhibited two alleles (*100* and *95*) and *PEP-B2* three alleles (*100*, *95* and *105*) in adults and juveniles. Neither locus was scored in post-larvae as ontogenetic changes appeared to mask allelic variation during the early settlement stages (see page 10).

*PEP-D*, a dimer, stained as a single locus with three alleles (*100*, *105* and *95*). Adults, juveniles and post-larvae were scored at this locus although enzyme activity was weak in a large number of individuals.

Both SOD and GPI exhibited a single weakly polymorphic locus; *SOD* generating allele products *100*, *95* and *110*, and *GPI* products *100*, *120*, *150*, *80* and *60*. *GPI* was scored in all life-history stages larger than mid-sized larvae, however, significant *SOD* activity was lost in animals smaller than juveniles.

MDH exhibited two loci in tail muscle tissue. *MDH-1* was very weakly polymorphic with two alleles (*100* and *120*), *MDH-2* was considered monomorphic in the samples examined (n = 516). Both loci were scored in individuals larger than mid-sized phyllosoma.

The genetic bases for the observed banding configurations were not derived through progeny studies, as the prolonged larval stage in *Palinurids* made such studies impractical. Without direct evidence from specific matings these bases must remain hypothetical. The

genetic interpretations advanced, however, are supported by the heterozygote banding patterns matching those predicted from enzyme structure and by the generally close agreements between the observed proportions of phenotypes and those expected on the basis of Hardy-Weinberg equilibrium (Selander *et al*, 1970).

Screening activities concentrated on resolving the enzyme systems studied by Menzies and co-workers (Menzies, 1981; Menzies and Kerrigan, 1979). Five of their loci appeared to have been successfully reproduced; *LDH-1*, *PGM*, *PEP-B1*, *PEP-B2*, and *PEP-D*, as in general the numbers and relative frequencies of the allele products identified in the two studies matched. However, only two alleles were resolved at *LDH-1*, the more cathodal electromorph being missed or excluded in this study.

## 2) MICROGEOGRAPHIC VARIATION

Allele frequencies for platform legal, platform sub-legal, platform juveniles and edge sub-legal samples are given in Table 2. Pooled data broken down by sex are also included.

The pronounced differences in colour exhibited by platform and edge individuals led to speculation that discrete sub-populations existed within the Bermuda stock. Spatial heterogeneity was investigated through comparisons between sub-legal sized deep water lobsters from the platform edge and similarly sized individuals from platform waters (Figure 1). Allele frequencies for the two samples are presented in Table 2 under edgesub and platsub.

Significant departure from Hardy-Weinberg expectations were determined at *PEP-B2* for the edge sub-legal sample ($X^2 = 16.38$, 3 d.o.f., $P < 0.001$. Exact test; $P < 0.046$). Although an excess of homozygotes was found, the excess was only slight with less than two individuals in each class out of a total sample size of 84.

No significant differences between the two samples were found at any of the seven loci examined (no comparisons were made at *MDH-1* due to small sample sizes) and the sum $X^2$-value was also not significant (sum $X^2 = 13.20$, 12 d.o.f. $P > 0.05$). There is therefore no evidence for spatial heterogeneity within the Bermuda stock.

To investigate other potential sources of population substructuring within the stock, allele frequency composition of the two sexes and three platform size-classes were also compared. The pooled male sample was found to have a significant excess of homozygotes at *PEP-B1* ($X^2 = 36.16$. 1 d.o.f., $P < 0.001$. Exact test; $P = 0.027$). However, this excess was confined to less than one individual per homozygote class. Chi-square tests for heterogeneity revealed no significant differences between the two sexes at any locus and therefore no evidence for sex-linkage.

The allele frequencies for the different platform size-classes are presented in Table 2 under platleg, platsub and juvenile. There were no significant departures from Hardy-Weinberg expectations. Chi-square tests between the size-classes (summarised in Table 3) demonstrated significant heterogeneity at *SOD* ($P < 0.05$) and *PEP-B1* ($P < 0.001$). Pairwise comparisons between the three size-classes demonstrated no significant differences between platform legal and sub-legal animals. However, juveniles were differentiated from legal animals at *PEP-B1* ($X^2 = 6.78$, 1 d.o.f., $P < 0.01$) and from the sub-legal sample at *PEP-B1* ($X^2 = 11.93$, 1 d.o.f., $P <$

0.001) and *SOD* ($X^2$ = 9.12, 2 d.o.f., $P < 0.05$).

The significant results all involved comparisons with the juvenile size-class which was limited to a small sample size over all loci studied (n = 27-43; see Table 1). In addition, the majority of the size-class heterogeneity was attributable to differences at *PEP-B1* and there remains some uncertainty regarding the contribution of ontogenetic changes to the observed *PEP-B1* phenotypes (see page 10). Consequently, the evidence suggesting population substructure exists by size must remain, to some extent, equivocal.

3) MACROGEOGRAPHIC VARIATION

To determine the genetic relatedness of the Bermuda stock to Caribbean stocks, sexually mature animals from the Bermuda platform were compared with animals obtained from six localities in the Caribbean (Figure 2). Genotypic frequencies for *P. argus* from Elliott Key (Florida), Key West (Florida), Cancun (Mexico), Trinidad, Jamaica and the Virgin Islands at 5 loci were obtained from raw data presented in Menzies (1981). Allele frequencies for these samples are presented in Table 4 with the presumptive equivalent allele frequencies scored from the Bermuda platleg sample (5 loci: *LDH-1, PGM, PEP-B1, PEP-B2* and *PEP-D*). Relative migrations of gene products were not given in Menzies (1981) so alleles were matched by distance from the anode and labelled alphabetically (Menzies, personal communication).

In 4 out of the 5 loci compared, the same number of alleles were found in the two studies and the frequency of the most common allele in Bermuda fell within the range determined by Menzies (1981). However, the slowest *LDH-1* allele was not resolved in Bermuda and the common allele was more abundant than elsewhere.

A contingency chi-square test indicated considerable heterogeneity between the sub-populations (Table 5). In addition, all pairwise comparisons between Bermuda and the six Caribbean sub-populations were statistically significant (sum $X^2$ range 47.00 - 109.86, 9 d.o.f.) with differences found at all five loci.

Although these results would appear to indicate considerable spatial heterogeneity within the global distribution of *P. argus*, they must be viewed with considerable caution as a number of assumptions have been made:

i) Menzies' samples were collected over ten years ago and there is currently no information available on temporal stability of allele frequencies at these sites (Walsh, personal communication).

ii) The five loci were assumed to be the same, but there is some uncertainty over the identity of the alleles. Allele products were matched by their positions relative to the anode (Menzies, personal communication), however different laboratory practices may have resulted in clearer resolution of alternative electromorphs.

To offset these uncertainties, specimens were obtained from two contemporary Caribbean sub-populations; Miami and the Florida Keys west of Marathon (Figure 2). Although these south Florida stocks were not obtained from the same sampling sites used by Menzies (Elliott Key and Key West), they were from the same general geographical areas.

Allele frequencies for the Miami and Florida Keys samples are presented in Table 4. Neither sample deviated significantly from Hardy-Weinberg expectations at any locus and a chi-square test determined the two stocks to be genetically homogeneous (sum $X^2 = 7.27$, 8 d.o.f., $P > 0.05$). Menzies' also determined the south Florida sub-populations to comprise a single stock as Elliott Key and Key West were undifferentiated (Menzies, 1981).

When the four Florida samples were analysed together (using the same five loci as before) considerable heterogeneity was recorded at *LDH, PEP-B1* and *PEP-B2* (Table 6), presumably as a result of temporal change, comparisons between different electromorphs or a combination of both. Comparisons between Bermuda and contemporary Caribbean sub-populations were therefore considered most meaningful. A chi-square test for heterogeneity failed to distinguish between the Miami, Florida Keys and Bermuda samples (Sum $X^2 = 25.47$, 20 d.o.f., $P > 0.05$), results consistent with a single Florida/western Atlantic lobster stock.

## 4) POST-LARVAL STUDIES

The source of recruits to Bermuda's lobster stock was examined through post-larval settlement and genetics studies.

Monthly post-larval settlement was calculated from data obtained from four 'Nomad' collector stations during 1990 and nine months of 1991. Monthly catch per unit effort (C.P.U.E.), calculated as the number of individuals settled per collector soak day, are displayed in Figure 3.

C.P.U.E. varied from 0.050 (s.d. = 0.085) in February 1991 to 1.864 (s.d. = 2.947) in August 1991. The large standard deviations recorded for each month are due to the monthly periodicity of post-larval influx, with maximum settlement occurring after the new moon and minimal settlement at full moon.

Settlement followed a similar pattern for both years, with a low-level incidence during winter months and highest influx in summer. This trend and periodic depressions in influx (e.g. July 1990) are typical of settlement in Bermuda in recent years (Ward, personal communication). Annual settlement, as measured by the total number of post-larvae removed from the four collector stations, varied by less than a factor of two in the last five years, from a minimum of 612 in 1989 to a maximum of 1081 in 1988. Thus recruitment to the Bermuda stock is currently stable.

The genetic structures of monthly post-larval samples were determined to enable comparisons to be made with the sexually mature components of the Bermuda and Caribbean stocks. The majority of the loci examined in the later benthic stages could be reproduced in post-larval specimens. However, *SOD* activity was lost and other systems appeared to exhibit non-allelic variation. Each locus was therefore tested for the continuity of allele products during the early stages of settlement. Post-larvae were staged according to the degree of pigmentation and the shape of the carapace; order of increasing settlement time being 'clear', 'partial', 'full' (all puerulus stages) and finally 'first stage juvenile'. Monthly samples were pooled for each stage and electromorph frequencies are presented in Table 7.

Four tests demonstrated significant deviation from Hardy-Weinberg expectations; three

at *PEP-B1* (clear; $X^2$ = 13.53, 1 d.o.f., P < 0.001; partial; $X^2$ = 26.88, 1 d.o.f., P < 0.001; full; $X^2$ = 4.15, 1 d.o.f., P < 0.05) and one at *PEP-B2* (1st stage juvenile; $X^2$ = 31.79, 1 d.o.f., P < 0.001).

Locus by locus chi-square tests for heterogeneity are summarised in Table 8. Two systems, *PEP-B1* and *PEP-B2* demonstrated significant heterogeneity by settlement stage. The *PEP-B1 '95'* band decreased in frequency with settlement time and the *PEP-B2 '100'* and *'105'* band frequencies increased dramatically at metamorphosis from the puerulus stage to first stage juvenile.

The highly significant deviations from equilibrium and the pronounced changes in electromorph frequencies suggest either strong selection for certain peptidase products or ontogenetic changes at these loci. The absence of the *PEP-B2 '105'* band in all puerulus stages and the virtual absence of the *PEP-B1 '100'* band in clear post-larvae (one individual in 44) strongly suggests ontogenetic changes to be responsible in both cases. Unlike first stage juveniles, the puerulus is a non-feeding stage (Lyons, 1980). Major biochemical changes would therefore be expected to occur immediately after settlement as the individual shifts from internal to external resources. Similar ontogenetic changes in hepatopancreas peptidase activities have been reported in larval and post-larval white shrimp, *Penaeus setiferus* (Lovett and Felder, 1990 a,b).

In view of these findings *PEP-B1* and *PEP-B2* could not be included in the post-larval genetic analyses. In addition, all significant comparisons involving these loci, for any life-history stage, should be viewed with considerable caution (see page 8).

The post-larval allele frequencies are presented in Table 9.

Initially only two polymorphic loci were successfully resolved (*PGM* and *GPI*) but by November 1990, two more polymorphic loci were being scored (*LDH-1* and *PEP-D*). No sample deviated significantly from Hardy-Weinberg expectations.

Locus by locus pairwise comparisons between the Bermuda breeding population, represented by the platleg sample, and monthly post-larval samples are summarised in Table 10 for 1990 and 1991. Two comparisons were determined to be significant; October 1990 at *PEP-D* ($X^2$ = 7.23, 2 d.o.f., P < 0.05) and May/June 1991, also at *PEP-D* ($X^2$ = 8.68, 2 d.o.f. P < 0.05). Similar comparisons were conducted between the post-larval samples and the two contemporary Florida sub-populations, Miami and Florida Keys. No significant differences were found (results not shown).

A total of 37 individual tests were conducted in the comparisons between Bermuda stock and post-larval samples, with just two significant results at P < 0.05. On the basis of chance alone, approximately two tests would be expected to be significant at this level. Therefore, there is no convincing evidence for the post-larval samples being genetically differentiated from either the Bermuda or Florida stocks during the course of this study.

## 5) PHYLLOSOMA STUDIES

The details of plankton tows and larval catches are summarised in Table 11. Monthly

tows were planned but weather and boat serviceability problems precluded a number of trips.

The nearshore catch was dominated by small phyllosoma (stages I and II) during the summer months, whereas mid to large-sized larvae were generally caught at very low frequencies in the offshore tows throughout the investigative period. One notable exception occurred in September 1991 when 187 mid-large phyllosoma were caught in a series of eight tows.

Genetic comparisons involving larval stages were not possible as a number of loci either could not be resolved or displayed weak activity. *PEP-D, PEP-B1, LDH-1* and *PGM* were unscorable in all phyllosoma and *GPI, PEP-B2, MDH-1* and *MDH-2* could only be scored in mid-large phyllosoma. As these loci either exhibited ontogenetic shifts, were monomorphic or only weakly polymorphic, comparisons with other larval or life-history stages were impractical.

## 6) EDDY ACTIVITY

Analyses of NOAA oceanographic frontal sheets demonstrated two warm-water outbreaks to have approached Bermuda inshore waters in 1990. Eddy 'A' arrived within 60 nautical miles of Bermuda on 9th April and was absorbed shortly after 25th May. Eddy 'B' converged before 5th June and dominated until approximately 7th July (Figure 4).

Two plankton sampling trips were arranged to strategically sample eddy 'B' north of Bermuda in June/July and details of the trips are summarised in Table 11. Thermometer readings of surface water temperature were unable to distinguish eddy core, sargasso and frontal waters. Similar strategic sampling of eddy waters in Australia were conducted with the aid of expendable bathythermographs (McWilliam and Phillips, 1983). As these facilities were not available and the NOAA satellite maps were of coarse resolution, strategic sampling of eddy waters had to be abandoned in favour of transect sampling through the water types.

The three plankton tows completed on the June voyage failed to produce *P. argus* phyllosoma and only two mid-sized larvae were caught in the July offshore tows. If warm-water outbreaks and eddies are vectors by which Caribbean larvae are deposited near Bermuda, the phyllosoma would be expected to be associated with the frontal water, as this originates from the Gulf Stream (Parker, 1971, McWilliam and Phillips, 1983). Conceivably, all five offshore tows missed the frontal waters or significant numbers of *P. argus* larvae were not associated with this water system. Tows 3 and 4 of the July voyage were conducted at the edge of, and upon, the Bermuda platform respectively and the large numbers of small phyllosoma were identified as stages I and II. Farmer *et al* (1989) have previously noted the preponderance of early-stage larvae from nearshore waters in July and their presence was probably unrelated to eddy proximity.

One warm-water outbreak approached to within 100 nautical miles of Bermuda in 1991; eddy 'C' converged before June 25th and had dissipated by July 11th (Figure 4). Unfortunately "R/V Calamus" was not available during this period for offshore plankton tow.

## DISCUSSION

The validity of many of the results and conclusions generated from this investigation are largely dependent on the compatibility of the Bermudian and Caribbean allozyme studies (Menzies and Kerrigan, 1979; Menzies, 1981). Problems and assumptions are an intrinsic part of comparative studies, particularly those involving different workers and non-overlapping investigative periods. Potential areas of incompatibility involving these two investigations and efforts at remedial action are discussed below.

1) Allelic identity. Menzies (1981) only identified his alleles by numbers and provided no information on the relative mobilities of the gene products. Obviously this has implications when matching alleles and comparing genotypic frequencies. Fortunately, information was provided on the relative positions of each allele product with reference to the anode (Menzies, personal communication).

The same number of alleles were detected at *PGM* (also confirmed by isoelectric focusing; Espinosa *et al*, 1990), *PEP-B1, PEP-B2* and *PEP-D* (Menzies, 1981). However, only two alleles were identified at *LDH-1* in this study, the slowest allele product not being found in any Bermuda samples. Contemporary samples obtained from south Florida sub-populations, where Menzies detected the slow allele at reasonably high frequencies, did exhibit a slow band (*'30'*) in a few individuals. However, as the heterozygote banding patterns did not conform to those predicted by the enzyme structure, the variation was not considered to have an allelic basis. Although three allele products were detected at *PEP-B2* in both studies, the relative frequencies differed. The fast band 'A' was more common than slow band 'C' in this study whereas Menzies found the reverse to be true. Therefore, despite modifications in tissue preparation and electrophoresis being kept to a minimum, minor changes in methodology may have resulted in different electromorphs being visualised at these two loci.

2) Temporal stability of allele frequencies in the Caribbean. Menzies' data is over ten years old and the genetic composition of these stocks may have altered with time. Contemporary samples obtained from two Florida sub-populations demonstrated temporal stability at two loci, *PGM* and *PEP-D*, but significant differences at *PEP-B1, PEP-B2* and *LDH-1.* However, the uncertainty regarding electromorph identity at *PEP-B2* and *LDH-1* means these differences cannot be unequivocally attributed to temporal effects.

3) Sample sizes. Three of the Caribbean samples contained less than fifty individuals (Cancun, Trinidad and the Virgin Islands) and for some loci only 20-30 individuals were scored (Menzies, 1981). It is possible that some of the observed heterogeneity within the Caribbean, and between these sites and Bermuda, could be artefacts due to small sample sizes. This cannot explain all observed heterogeneity, however, as sample sizes from the remaining three Caribbean sites were reasonable, yet differences were recorded at all loci.

In view of these compatibility problems, particularly the concerns regarding allelic identity, the two studies will be considered separately, with conclusions restricted to the results obtained from contemporary analyses.

This study failed to demonstrate convincing evidence for population substructuring within

the Bermuda stock nor significant heterogeneity within the limited spatial distribution of adult stocks examined (Bermuda and south Florida). As Menzies (1981) also could not distinguish between south Florida sub-populations, the combined results are consistent with a single Florida/western Atlantic lobster stock. These results contrast with similar genetic studies conducted on molluscs; Bermuda queen conch (*Strombus gigas*) were genetically differentiated from a relatively homogeneous Caribbean (Mitton, Berg and Orr, 1989) and the calico scallop (*Argopecten gibbus*) was sufficiently different from U.S. populations to be considered a distinct species (Krausse, personal communication). However, the planktonic residence times for the larvae of these two species (approximately two to four weeks) are considerably less than that estimated for *P. argus*, with a proportional reduction in larval dispersal capability.

The genetic relatedness of *P. argus* stocks elsewhere within the species' distribution are uncertain, as data from a number of studies are conflicting. Menzies' allozyme studies demonstrated considerable heterogeneity within the Caribbean, explained through predominantly local recruitment and post-settlement selection (Menzies, 1981; 1991). Demonstrable sub-population differentiation within the Family *Palinuridae* would therefore be restricted to *P. argus* as protein polymorphism studies of the related species *P. ornatus* (Salini *et al*, 1985), *P. marginatus* (Shaklee and Samollow, 1984) and *Jasus edwardsii* (Smith, McKoy and Machin, 1980) all failed to differentiate geographically separated samples. In contrast, an allozyme study of two Brazilian *P. argus* sub-populations theoretically isolated by current differences (South equatorial and Brazilian) were genetically very similar (Ogawa *et al*, 1990). Similarly, phyllosomal larvae collected in Cuban waters were found to be genetically homogeneous when compared by developmental stage and sampling station (Espinosa, Diaz and Berovides, 1990).

*P. argus* mitochondrial DNA exhibits high levels of heterogeneity but results from restriction fragment length polymorphism (RFLP) studies are currently limited. Reports of local races in Florida populations (McLean, Okubo and Tracey, 1983; Komm *et al*, 1982) should be viewed with considerable caution as sample sizes were extremely small (varying from 1 to 20 in both cases). Preliminary results from a large study based at the University of Miami indicate a number of stocks distributed throughout the Caribbean to be genetically homogeneous (Silberman, personal communication).

Assuming *P. argus* can be found in geographically differentiated stocks, what forces could maintain heterogeneity? Menzies argued that absolute isolation of populations was unlikely as only a small number of foreign settlers are necessary to eliminate the differentiating effects of drift (estimated as 1 in 10-100,000 recruits per generation). Local selection pressures were therefore considered responsible for maintaining differentiation (Menzies, 1981; 1991).

Pre-settlement selection could not be tested due to problems resolving suitable enzyme systems in encapsulated larvae and phyllosomal stages. Evidence for post-settlement selection was weak; declines in the relative frequencies of the *PEP-B1 95* and *PEP-B2 105* alleles from juveniles to sub-adults (Table 2), and the rare *GPI 80* allele being found in post-larvae but not in the resident stock (Table 9). In the former cases, small juvenile sample size and differences at loci that exhibit ontogenetic shifts during early settlement prevented unequivocal interpretation for selection. Also, the frequency of the *PEP-B1 95* allele in first stage juveniles (Table 7) was comparable to that found in sub-adults. In the latter case, the *80* allele was sufficiently rare to have been missed by chance in analyses of Bermuda samples. The presence of a rare esterase allele in the Elliott Key post-larval sample initially considered as evidence for post-settlement

selection (Menzies and Kerrigan, 1979) was later refuted when the same allele appeared in the adult population (Menzies, 1981). Pollock (1990) has proposed that speciation in palinurid lobsters may gradually occur through a combination of post-settlement mortality and selection for phyllosoma larvae with highly specific recognitive faculties which trigger metamorphosis into the post-larval stage. Foreign arrivals would therefore be individuals that erred in cue identification. However, at this stage evidence for post-settlement selection is limited and discriminatory larval metamorphosis behaviour remains hypothetical.

Monthly post-larval samples were analysed in order to further investigate the source of recruits to the Bermuda stock. As most samples were undifferentiated from the Bermuda breeding population and in equilibrium, the input from genetically differentiated stocks was considered to be negligible. These results, combined with the lack of convincing evidence for within-stock substructure, initially appear consistent with local replenishment being the primary recruitment mechanism during the investigative period. However, as the monthly recruits were also genetically indistinguishable from the two contemporary Florida samples, distant stocks with similar genetic composition may also have had a significant input.

The Bermuda population has a single breeding season in the summer months (Sutcliffe, 1952), yet post-larval recruitment occurs throughout the year, with peak influx also occurring in summer (Figure 3; Ward, personal communication). Farmer *et al* (1989) proposed peak settlement coincided with the arrival of locally derived lobsters, whereas the low-level incidence during winter could either be explained through foreign arrivals, or local larvae exhibiting plasticity in development times. Florida populations undergo peak spawning activity in May/June (Lyons, 1981), therefore the larvae would have to complete development within 7-8 months if they were to be responsible for winter settlement in Bermuda. This would be at the lower end of the scale estimating *P. argus* larval duration (Sims and Ingle, 1967) but probably feasible given faster growth rates during the early stages of development whilst in the warm waters of the Gulf Stream (Austin, 1972; Farmer, Ward and Luckhurst, 1989; Chittleborough and Thomas, 1969). Delayed development times have been noted for *P. cygnus* (Phillips and McWilliam, 1986) and proposed for *Jasus edwardsii* (Booth, 1990). As this cannot be dismissed as a factor in *P. argus* larval development, the relative contributions of non-Bermudian and locally-derived larvae to the Bermuda stock cannot be determined.

The role of Gulf Stream warm-water outbreaks in larval transport and post-larval recruitment to Bermuda also remains uncertain. Mesoscale eddies have been demonstrated to be vectors for phyllosomal larvae in Australian waters (McWilliam and Phillips, 1983) and phyllosoma from Caribbean stocks may be expected to be associated with Atlantic eddy frontal waters. If so, post-larval influx could be influenced by eddy proximity in two ways; settlement rates and genetic composition.

Comparisons of influx rates between weeks when eddies were within a certain distance of Bermuda with those when they were absent would be affected by the monthly periodicity of settlement (Ward, unpublished results; Menzies, 1981). Similarly, monthly comparisons would be disturbed by annual settlement patterns (Figure 3). As the time period from larvae arriving in Bermuda's offshore waters via Gulf Stream waters to metamorphosis/settlement presumably will also vary, only comparisons between years would be unbiased. Annual settlement data has been collected in Bermuda for the last six years (1985-1990), however, the equivalent satellite data for sea surface temperatures are currently not available for much of this period.

Two eddies impinged on Bermuda's nearshore waters in April and June 1990 and one approached to within 60 nautical miles in June/July 1991 (Figure 4). Although few loci were available for discriminating comparisons in 1990 (eddies 'A' and 'B'), more polymorphic loci were available for June/July 1991 (eddy 'C'). Post-larvae sampled during, and shortly after, these periods were genetically indistinguishable from the Bermuda stock, thus recruitment from eddy larval stocks remains unproven.

Management of the Bermuda lobster stock has been based on the assumption that it is a self-replenishing resource. The crop is currently protected through a minimum legal size (92mm carapace length), five month closed season, the release of gravid females, limited commercial entry, gear restrictions and limited recreational harvest. As post-larval recruitment has been stable over the last five years, the fishery has supported an annual catch of approximately 30,000 individuals for fifteen years and genetic analyses of benthic and post-larval individuals are consistent with a hypothesis of local replenishment, these management policies appear to be appropriate.

However, the inability to distinguish south Florida and Bermuda sub-populations suggests these stocks actually constitute a single wider Florida/western Atlantic stock. Management efforts should therefore be co-ordinated on a regional basis with Florida State and Federal Authorities (through the South Atlantic Fishery Management Council and the Department of Natural Resources). Too little information is currently available to accurately delineate the management unit. Preliminary work by Menzies and Kerrigan (1979) suggested Bahamas and Florida stocks were also closely related, thus major co-operation with the Bahamas would increase the probability of successful resource management (Menzies, 1991). The Florida/western Atlantic stock therefore appears to be distributed over approximately 1000 nautical miles. This scale is not unusual for *Palinurids* as the Hawaiian islands (in excess of 1500 miles in length) constitute a single stock for *P. marginatus* (Shaklee and Samollow, 1984) and the wide distributions of *P. cygnus* and *P. ornatus* are also managed as single stocks (Phillips and McWilliam, 1986; Salini *et al*, 1985).

The results from this study support closer international co-operation in the management of *P. argus* resources. A pan-Caribbean management plan is recommended, but political constraints suggest implementation would be difficult. Additional information on stock distributions within the Caribbean would therefore be required for effective management on a regional basis, although countries with shared or contiguous fisheries such as i) Mexico and Belize. ii) Honduras and Nicaragua, and iii) Grenada, St. Vincent and the Grenadines are already in a position to implement co-operative management (Villegas, Jones and Labisky, 1982). Similar recommendations are applicable to other trans-boundary spiny lobster fisheries. Species supporting significant fisheries in more than one country include; *P. homarus* (S.E. Asia/Indian Ocean/Australia), *P. interruptus* (Mexico and the U.S.A.), *P. guttatus* and *P. laevicauda* (Caribbean and S. America) and *Jasus verreauxi* (Australia and New Zealand) Williams, 1988).

## FINANCE

There were no significant problems with project financing and the study was completed within the budgetary framework. A detailed account of financial expenditure is not available from the investigator as many invoices were forwarded directly from the suppliers to Southampton University.

## APPENDIX 1: EXPERIMENTS WITH COLLECTOR DESIGNS

To supplement post-larval collecting capacity, the four Nomad collectors originally deployed by the Division of Fisheries were supplemented by twelve extra collectors; four Nomad dype, four Calinsky type and four DNR type (the specifications for each design are available from Bermuda Government, Division of Fisheries). To test the relative efficacies of each design, one collector of each type was deployed in four identical arrays along the North Shore (Figure 1).

After approximately 18 months deployment and 290 visits to each collector type, the mean catch per unit efforts were; Nomad 0.395 (s.d. = 0.794); DNR 0.426 (s.d. = 0.892) and Calinsky 0.347 (s.d. = 0.694). One-way analysis of variance failed to demonstrate any significant difference between collector designs ($F = 0.73$, 2 d.o.f., $P > 0.05$).

To maximise post-larval collections during winter months, additional collectors were therefore deployed rather than replacing the existing Fisheries Nomad design with a better collector. Although materials and construction costs were higher for Nomad collectors, superior durability, longevity and sampling ease supports their retention as the settlement monitoring habitat.

## APPENDIX 2: IDENTIFICATION OF PHYLLOSOMA BY ALLOZYME ELECTROPHORESIS

Allozyme electrophoresis was particularly useful as a means of distinguishing *P. argus* phyllosoma from other lobster larvae. When 70 individuals from the September 1991 catch were analysed, 46 (66%) generated banding patterns of type 'A', 23 (33%) produced type 'B' and 1 (1%) produced type 'C'. Type 'A' patterns were identical to those produced by *P. argus* pueruli at *GPI, PEP-B2, MDH-1* and *MDH-2*. Type 'B' larvae displayed a single polymorphic GPI locus (sharing electromorphs with *P. argus*), a single polymorphic MDH locus and three PEP-B loci. These larvae were later identified as *P. guttatus* by mitochondrial DNA RFLP methods (Silberman, personal communication). Type 'C' larvae also displayed a single GPI locus with a distinct electromorph from types 'A' and 'B', and from morphological criteria was identified as belonging to the Family *Scyllaridae*. Allele frequencies of the three types are displayed in Table 12, demonstrating the value of GPI, PEP-B and MDH as distinguishing and diagnostic systems.

Of an additional 63 samples sent to Silberman at the University of Miami for mtDNA analysis, 56 were identified as *P. argus* (89%) and 7 as *P. guttatus* (11%). The large disparity in the relative proportions of *P. guttatus* determined by the two methods was due to the largest larvae generally being *P. guttatus* (Silberman, personal communication) and these being selected out for the allozyme study. Pooling the two studies produced the following numbers and proportions; *P. argus*, 102 individuals (77%); *P. guttatus*, 30 individuals (22%); *Scyllaridae*, 1 individual (1%).

Attempts to verify the origin of a stock through the distribution of phyllosoma in major or localised currents in the Caribbean have often been frustrated by an inability to distinguish between larvae of the three *Panulirus* species which occur in the region; *P. argus, P. guttatus* and *P. laevicauda* (Lyons, 1986; Austin, 1972, Sims and Ingle, 1967). As *P. argus* tends to be the most abundant lobster in the region, researchers often assume all *Panulirus* larvae are of this species (Sims and Ingle, 1967; Farmer, Ward and Luckhurst, 1989). Allozyme electrophoresis is therefore a useful method for estimating the relative frequencies of *P. argus* and *P. guttatus* in a sample. Unfortunately, as specimen preparation is destructive, potentially discriminating morphometric information cannot easily be obtained. DNA analysis is superior in this respect as one leg can provide all the tissue required for analysis, the remainder of the animal being available for morphometrics (Silberman, personal communication).

## REFERENCES


Austin, H.M., 1972. "Notes on the distribution of phyllosoma of the spiny lobster, *Panulirus* ssp., in the Gulf of Mexico". - Proc. Natl. Shellfisheries Assoc. **62:** 26-30.

Booth, J.D., 1990. "Occurrence of mid- and late-stage phyllosoma larvae, and pueruli, of the rock lobster *Jasus edwardsii* in waters near Gisborne, New Zealand, in relation to puerulus settlement onshore". - Abstracts of International Workshop on Lobster Ecology and Fisheries, Havana, Cuba, June 12-16, 1990.

Chittleborough, R.G., and L.R. Thomas, 1969. "Larval ecology of the Western Australian marine crayfish with notes upon other panulurid larvae from the eastern Indian Ocean". - Aust. J. Mar. Freshw. Res. **26:** 317-328.

Espinsoa, G., R. Diaz and V. Berovides, 1990. "Analisis genetico bioquimico de larvas de *Panulirus argus* de la zona economica exclusiva del sur de Cuba". - Abstracts of International Workshop on Lobster Ecology and Fisheries, Havana, Cuba, June 12-16, 1990.

Espinosa, G., E. Wenisch, H. Danner, H. Katinger and P.G. Righetti, 1990. "Isoelectric focusing in immobilized pH gradients of phosphoglucomutase and esterases from the spiny lobster". - Electrophoresis **11:** 810-812.

Farmer, M.W. and C.J. Berg, 1989. "Circulation around islands, gene flow, and fisheries management". - Proc. Gulf Carib. Fish. Inst. **39:** 318-330.

Farmer, M.W., J.A. Ward and B.E. Luckhurst, 1989. "Development of spiny lobster (*Panulirus argus*) phyllosoma larvae in the plankton near Bermuda". - Proc. Gulf Carib. Fish. Inst. **39:** 289-301.

Harris, H. and D.A. Hopkinson, 1976. "Handbook of enzyme electrophoresis". - North Holland Elsevier Pub. Co. Inc., New York.

Johnson, M.W., 1960. "The offshore drift of larvae of the California spiny lobster *Panulirus interruptus*". - Calif. Coop. Ocean. Fish. Invest. Rep. **7:** 147-161.

Komm, B., A. Michaels, J. Toskos and J. Linton, 1982. "Isolation and characterisation of the mitochondrial DNA from the Florida spiny lobster, *Panulirus argus*". - J. Comp. Biochem. Physiol. **78B:** 923-929.

Lewis, J.B., 1951. "The phyllosoma larvae of the spiny lobster *Panulirus argus*". - Bull. Mar. Sci. Gulf Carib. **1:** 89-103.

Lovett, D.L. and D.L. Felder, 1990a. "Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae)". - Biol. Bull. **178:** 144-159.

Lovett, D.L. and D.L. Felder, 1990b. "Ontogenetic changes in enzyme distribution and midgut formation in developmental stages of *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae)". - Biol. Bull. **178:** 160-174.

Lyons, W.G., 1980. "The post-larval stage of *Scyllaridean* lobsters". - Fisheries **5:** 48.

Lyons, W.G., 1981. "Possible sources of Florida's spiny lobster population". - Proc. Gulf Carib. Fish. Inst. **33:** 253-266.

Lyons, W.G., 1986. "Problems and perspectives regarding recruitment of spiny lobsters, *Panulirus argus*, to the south Florida fishery". - Can. J. Fish. Aquat. Sci. **43:** 2099-2106.

McLean, M., C.K. Okubo and M.L. Tracey, 1983. "MtDNA heterogeneity in *Panulirus argus*". - Experientia **39:** 536-538.

McWilliam, P.S. and B.F. Phillips, 1983. "Phyllosoma larvae and other crustacean macrozooplankton assocated with eddy J, a warm core eddy off south-eastern Australia". - Aust. J. Mar. Freshw. Res. **34:** 653-663.

Menzies, R.A. and J.M. Kerrigan, 1979. "Implications of spiny lobster recruitment patterns of the Caribbean - a biochemical genetic approach". - Proc. Gulf Carib. Fish. Inst. **31:** 164-178.

Menzies, R.A., 1981. "Biochemical population genetics and the spiny lobster recruitment problem: an update". - Proc. Gulf Carib. Fish. Inst. **33:** 230-243

Menzies, R.A., 1991. "Allozyme variation and conservation: applications to spiny lobsters and crocodilians". - In, "Species conservation: a population-biological approach". (Eds. Seitz, A. and V. Loeschcke). Birkhauser Verlag, Basel. In press.

Mitton, J.B., C.J. Berg and K.S. Orr, 1989. "Population structure, larval dispersal, and gene flow in the queen conch, *Strombus gigas*, of the Caribbean". - Biol. Bull. **177:** 356-362.

Ogawa, M., G.M. Oliveira, K. Sezaki, S. Watabe and K. Hashimoto, 1990. "Genetic variation in the spiny lobsters, *Panulirus argus, Panulirus laevicauda* and *Panulirus japonicus*". - Abstracts of International Workshop on Lobster Ecology and Fisheries, Havana, Cuba, June 12-16, 1990.

Parker, C.E., 1971. "Gulf Stream rings in the Sargasso Sea". - Deep Sea Res. **18:** 981-993.

Phillips, B.F., 1981. "The circulation of the south-eastern Indian Ocean and planktonic life of the western rock lobster". - Ocean. Mar. Biol. Annu. Rev. **19:** 11-39.

Phillips, B.F. and P.S. McWilliam, 1986. "The pelagic phase of spiny lobster development". - Can. J. Fish. Aquat. Sci. **43:** 2153-2163.

Pollock, D.E., 1990. "Palaeoceanography and speciation in the spiny lobster genus *Jasus*". - Bull. Mar. Sci. **46:** 387-405.

Salini, J., G. Rintoul, J. Shaklee and B. Phillips, 1985. "An electrophoretic analysis of stock structure in the ornate tropical rock lobster, *Panulirus ornatus*". - In, Torres Strait Fisheries Seminar (eds. Haines, A.K. and G.C. Williams). Australian Government Publishing Service, Canberra. 218-232.

Selander, R.B., S.Y. Yang, R.C. Lewontin and W.E. Johnson, 1970. "Genetic variation in the horeshoe crab (*Limulus polyphemus*), a phylogenetic 'relic'". - Evolution **24:** 402-414.

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. Natn. Ocean. Atmos. Admin. **82:** 693-702.

Shaw. C.R. and R. Prasad, 1970. "Starch gel electrophoresis of enzymes - a compilation of recipes". - Biochem. Gen. **4:** 297-320.

Sims. H.W. and R.M. Ingle, 1967. "Caribbean recruitment of Florida's spiny lobster population". - Quart. J. Florida Acad. Sci. **29:** 207-242.

Smith, P.J., J.L. McKoy and P.J. Machin, 1980. "Genetic variation in the rock lobsters *Jasus edwardsii* and *Jasus novaehollandiae*". - N.Z. J. Mar. Freshw. Res. **14:** 55-63.

Sutcliffe, W.H., 1952. "Some observations of the breeding and migration of the Bermuda spiny lobster, *Panulirus argus*". - Proc. Gulf Carib. Fish. Inst. **4:** 64-69.

Swofford D.L., and R.B. Selander, 1981. "BIOSYS-1: A fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics". - J. Hered. **72:** 281-283.

Villegas, L., A.C. Jones and R.F. Labisky, 1982. "Management strategies for the spiny lobster resources in the western central Atlantic: a cooperative approach". - N. Amer. J. Fish. Manage. **2:** 216-223.

Ward, J., 1989. "Patterns of settlement of spiny lobster (*Panulirus argus*) post-larvae at Bermuda". - Proc. Gulf Carib. Fish. Inst. **39:** 255-264.

Williams, A.B., 1988. "Lobsters of the world. An illustrated guide". - Osprey Books, Huntington. 186 pp.

Witham, R., R.M. Ingle and E. Joyce, 1968. "Physiological and ecological studies of *Panulirus argus* from St. Lucie estuary". - Florida Board Conserv. Tech. Ser. 53.

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TABLE 1

SUMMARY OF SCREENING EXPERIMENTS FOR ENZYME ACTIVITY

A = adult & sub-adult, J = juvenile, PL = post-larvae, PH = phyllosoma

| ENZYME SYSTEM | ACTIVITY | TISSUE USED IN ANALYSIS |
|---|---|---|
| ACP | 2 loci, *ACP-1* polymorphic | Leg (A, J) |
| | *ACP-2* monomorphic | Leg (A, J) |
| EST | Unreadable | |
| FH | No activity | |
| GLUD | No activity | |
| GPI | 1 locus, polymorphic | Leg (A, J), Tail (PL), Whole (PH) |
| HK | No activity | |
| IDH | Unreadable | |
| LDH | 2 loci, *LDH-1* polymorphic | Leg (A, J, PL) |
| | *LDH-2* monomorphic | Leg (A, J) |
| MDH | 2 loci, *MDH-1* polymorphic | Tail (A, J, PL), Whole (PH) |
| | *MDH-2* monomorphic | Tail (A, J, PL), Whole (PH) |
| ME | 1 locus, monomorphic | Tail (A, J, PL) |
| ODH | No activity | |
| PEP-B | 2 loci, *PEP-B1* polymorphic | Hepatopancreas (A, J), Cephalothorax (PL), Whole (PH) |
| | *PEP-B2* polymorphic | Hepatopancreas (A, J), Cephalothorax (PL), Whole (PH) |
| PEP-D | 1 locus, polymorphic | Hepatopancreas (A, J), Cephalothorax (PL) |
| PGM | 1 locus, polymorphic | Leg (A, J), Tail (PL) |
| SDH | No activity | |
| 6PGDH | No activity | |
| SOD | 1 locus, polymorphic | Leg (A, J) |
| XDH | No activity | |

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| FH | No activity | |
| GLUD | No activity | |
| GPI | 1 locus, polymorphic | Leg (A, J), Tail (PL), Whole (PH) |
| HK | No activity | |
| IDH | Unreadable | |
| LDH | 2 loci, *LDH-1* polymorphic | Leg (A, J, PL) |
| | *LDH-2* monomorphic | Leg (A, J) |
| MDH | 2 loci, *MDH-1* polymorphic | Tail (A, J, PL), Whole (PH) |
| | *MDH-2* monomorphic | Tail (A, J, PL), Whole (PH) |
| ME | 1 locus, monomorphic | Tail (A, J, PL) |
| ODH | No activity | |
| PEP-B | 2 loci, *PEP-B1* polymorphic | Hepatopancreas (A, J), Cephalothorax (PL), Whole (PH) |
| | *PEP-B2* polymorphic | Hepatopancreas (A, J), Cephalothorax (PL), Whole (PH) |
| PEP-D | 1 locus, polymorphic | Hepatopancreas (A, J), Cephalothorax (PL) |
| PGM | 1 locus, polymorphic | Leg (A, J), Tail (PL) |
| SDH | No activity | |
| 6PGDH | No activity | |
| SOD | 1 locus, polymorphic | Leg (A, J) |
| XDH | No activity | |

TABLE 2

ALLELE FREQUENCIES FOR SIZE-CLASS SAMPLES AND POOLED SAMPLES BROKEN DOWN BY SEX.
N = Number of individuals

| LOCUS | ALLELE | PLATLEG | PLATSUB | EDGESUB | JUVENILE | MALE | FEMALE |
|---|---|---|---|---|---|---|---|
| *LDH-1* | N | 61 | 83 | 84 | 43 | 142 | 127 |
| | *100* | 0.984 | 0.994 | 0.988 | 1.000 | 0.996 | 0.984 |
| | *110* | 0.016 | 0.006 | 0.012 | 0.000 | 0.004 | 0.016 |
| *SOD* | N | 57 | 76 | 61 | 39 | 118 | 110 |
| | *100* | 0.974 | 0.993 | 0.992 | 0.924 | 0.970 | 0.982 |
| | *110* | 0.026 | 0.000 | 0.008 | 0.038 | 0.021 | 0.009 |
| | *95* | 0.000 | 0.007 | 0.000 | 0.038 | 0.009 | 0.009 |
| *PGM* | N | 60 | 83 | 83 | 40 | 141 | 123 |
| | *100* | 0.975 | 0.970 | 0.958 | 0.963 | 0.958 | 0.976 |
| | *200* | 0.008 | 0.030 | 0.036 | 0.025 | 0.028 | 0.024 |
| | *-200* | 0.017 | 0.000 | 0.006 | 0.012 | 0.014 | 0.000 |
| *PEP-B1* | N | 60 | 63 | 83 | 39 | 128 | 112 |
| | *100* | 0.975 | 0.992 | 0.994 | 0.885 | 0.973 | 0.982 |
| | *95* | 0.025 | 0.008 | 0.006 | 0.115 | 0.027 | 0.018 |
| *PEP-B2* | N | 35 | 52 | 84 | 43 | 114 | 98 |
| | *100* | 0.886 | 0.846 | 0.928 | 0.756 | 0.860 | 0.872 |
| | *105* | 0.114 | 0.115 | 0.054 | 0.209 | 0.123 | 0.097 |
| | *95* | 0.000 | 0.039 | 0.018 | 0.035 | 0.017 | 0.031 |
| *PEP-D* | N | 30 | 48 | 62 | 27 | 91 | 76 |
| | *100* | 0.633 | 0.573 | 0.621 | 0.630 | 0.643 | 0.572 |
| | *95* | 0.300 | 0.396 | 0.371 | 0.352 | 0.330 | 0.395 |
| | *105* | 0.067 | 0.031 | 0.008 | 0.018 | 0.027 | 0.033 |
| *GPI* | N | 62 | 83 | 84 | 43 | 142 | 64 |
| | *100* | 1.000 | 0.982 | 1.000 | 1.000 | 0.989 | 1.000 |
| | *60* | 0.000 | 0.012 | 0.000 | 0.000 | 0.007 | 0.000 |
| | *120* | 0.000 | 0.006 | 0.000 | 0.000 | 0.004 | 0.000 |
| *MDH-1* | N | 2 | 40 | 5 | 16 | 31 | 32 |
| | *100* | 1.000 | 0.975 | 1.000 | 1.000 | 1.000 | 0.969 |
| | *120* | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.031 |

TABLE 3

LOCUS BY LOCUS CHI-SQUARE TEST FOR HETEROGENEITY AMONG BERMUDA PLATFORM SIZE-CLASSES

| LOCUS | $X^2$-VALUE | d.o.f. | PROBABILITY |
|---|---|---|---|
| *LDH-1* | 1.85 | 2 | $P > 0.05$ |
| *SOD* | 11.90 | 4 | $P < 0.05$ |
| *PGM* | 4.16 | 4 | $P > 0.05$ |
| *PEP-B1* | 15.57 | 2 | $P < 0.001$ |
| *PEP-B2* | 6.97 | 4 | $P > 0.05$ |
| *PEP-D* | 3.20 | 4 | $P > 0.05$ |
| *GPI* | 3.83 | 4 | $P > 0.05$ |
| SUM | 47.78 | 24 | $P < 0.01$ |

TABLE 4

ALLELE FREQUENCIES FOR BERMUDA AND CARIBBEAN SAMPLES.
N = Number of individuals

| | | | | | ----------DATA FROM MENZIES (1981)---------- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| LOCUS | ALLELE | BERMUDA PLATLEG | MIAMI | FLORIDA KEYS | ELLIOTT KEY | KEY WEST | CANCUN | TRINIDAD | JAMAICA | VIRGIN ISLANDS |
| *LDH-1* | N | 61 | 39 | 31 | 50 | 58 | 37 | 29 | 47 | 37 |
| | *A* | 0.008 | 0.000 | 0.000 | 0.000 | 0.008 | 0.081 | 0.000 | 0.043 | 0.000 |
| | *B* | 0.992 | 1.000 | 1.000 | 0.840 | 0.897 | 0.865 | 0.948 | 0.914 | 0.703 |
| | *C* | 0.000 | 0.000 | 0.000 | 0.160 | 0.095 | 0.054 | 0.052 | 0.043 | 0.297 |
| *SOD* | N | 57 | 42 | 35 | | | | | | |
| | *100* | 0.974 | 0.988 | 1.000 | | | | | | |
| | *110* | 0.026 | 0.012 | 0.000 | | | | | | |
| *PGM* | N | 60 | 32 | 33 | 54 | 46 | 32 | 31 | 40 | 38 |
| | *A* | 0.008 | 0.047 | 0.045 | 0.028 | 0.098 | 0.062 | 0.000 | 0.038 | 0.092 |
| | *B* | 0.975 | 0.938 | 0.955 | 0.954 | 0.902 | 0.938 | 1.000 | 0.962 | 0.895 |
| | *C* | 0.017 | 0.015 | 0.000 | 0.018 | 0.000 | 0.000 | 0.000 | 0.000 | 0.013 |
| *PEP-B1* | N | 60 | 42 | 29 | 66 | 43 | 31 | 28 | 20 | 22 |
| | *A* | 0.975 | 1.000 | 1.000 | 0.833 | 0.802 | 0.694 | 0.929 | 0.500 | 1.000 |
| | *B* | 0.025 | 0.000 | 0.000 | 0.167 | 0.198 | 0.306 | 0.071 | 0.500 | 0.000 |
| *PEP-B2* | N | 35 | 35 | 31 | 58 | 43 | 29 | 25 | 44 | 30 |
| | *A* | 0.114 | 0.114 | 0.081 | 0.025 | 0.012 | 0.069 | 0.000 | 0.000 | 0.017 |
| | *B* | 0.886 | 0.843 | 0.919 | 0.897 | 0.756 | 0.759 | 0.620 | 0.591 | 0.917 |
| | *C* | 0.000 | 0.043 | 0.000 | 0.078 | 0.231 | 0.171 | 0.380 | 0.409 | 0.066 |
| *PEP-D* | N | 30 | 39 | 23 | 97 | 65 | 38 | 31 | 50 | 45 |
| | *A* | 0.067 | 0.026 | 0.000 | 0.026 | 0.015 | 0.013 | 0.097 | 0.030 | 0.000 |
| | *B* | 0.633 | 0.615 | 0.565 | 0.505 | 0.515 | 0.632 | 0.548 | 0.630 | 0.578 |
| | *C* | 0.300 | 0.359 | 0.435 | 0.469 | 0.470 | 0.355 | 0.355 | 0.340 | 0.422 |
| *GPI* | N | 62 | 40 | 35 | | | | | | |
| | *100* | 1.000 | 0.987 | 1.000 | | | | | | |
| | *120* | 0.000 | 0.013 | 0.000 | | | | | | |

TABLE 5

LOCUS BY LOCUS CHI-SQUARE TEST FOR HETEROGENEITY AMONG BERMUDA AND CARIBBEAN SUB-POPULATIONS (FROM MENZIES, 1981)

| LOCUS | $X^2$-VALUE | d.o.f. | PROBABILITY |
|---|---|---|---|
| *LDH-1* | 79.63 | 12 | $P < 0.001$ |
| *PGM* | 22.92 | 12 | $P < 0.05$ |
| *PEP-B1* | 74.12 | 6 | $P < 0.001$ |
| *PEP-B2* | 93.25 | 12 | $P < 0.001$ |
| *PEP-D* | 27.14 | 12 | $P < 0.01$ |
| SUM | 297.06 | 54 | $P < 0.001$ |

TABLE 6

LOCUS BY LOCUS CHI-SQUARE TEST FOR HETEROGENEITY AMONG SOUTH FLORIDA SUB-POPULATIONS, USING CONTEMPORARY DATA AND MENZIES' 1981 DATA

| LOCUS | $X^2$-VALUE | d.o.f. | PROBABILITY |
|---|---|---|---|
| *LDH-1* | 24.61 | 6 | $P < 0.001$ |
| *PGM* | 7.71 | 6 | $P > 0.05$ |
| *PEP-B1* | 29.01 | 3 | $P < 0.001$ |
| *PEP-B2* | 35.04 | 6 | $P < 0.001$ |
| *PEP-D* | 4.66 | 6 | $P > 0.05$ |
| SUM | 101.03 | 27 | $P < 0.001$ |

TABLE 7

ELECTROMORPH FREQUENCIES AT 6 LOCI FOR FOUR STAGES OF POST-LARVAE
N = Number of individuals

| LOCUS | BAND | PUERULI | | | 1ST STAGE JUVENILE |
|---|---|---|---|---|---|
| | | CLEAR | PARTIAL | FULL | |
| *LDH-1* | N | 65 | 98 | 79 | 186 |
| | *100* | 0.992 | 0.995 | 1.000 | 1.000 |
| | *110* | 0.008 | 0.005 | 0.000 | 0.000 |
| *PGM* | N | 81 | 93 | 132 | 207 |
| | *100* | 1.000 | 0.978 | 0.973 | 0.973 |
| | *200* | 0.000 | 0.022 | 0.011 | 0.022 |
| | *-200* | 0.000 | 0.000 | 0.015 | 0.005 |
| *PEP-B1* | N | 44 | 110 | 83 | 214 |
| | *100* | 0.398 | 0.464 | 0.488 | 0.960 |
| | *95* | 0.602 | 0.536 | 0.512 | 0.040 |
| *PEP-B2* | N | 59 | 105 | 77 | 97 |
| | *100* | 0.051 | 0.057 | 0.045 | 0.397 |
| | *95* | 0.949 | 0.943 | 0.955 | 0.567 |
| | *105* | 0.000 | 0.000 | 0.000 | 0.036 |
| *PEP-D* | N | 47 | 73 | 53 | 126 |
| | *100* | 0.479 | 0.527 | 0.528 | 0.560 |
| | *95* | 0.489 | 0.459 | 0.434 | 0.420 |
| | *105* | 0.032 | 0.014 | 0.038 | 0.020 |
| *GPI* | N | 81 | 133 | 93 | 215 |
| | *100* | 0.994 | 0.992 | 0.995 | 0.996 |
| | *80* | 0.000 | 0.008 | 0.005 | 0.002 |
| | *120* | 0.006 | 0.000 | 0.000 | 0.002 |

TABLE 8

LOCUS BY LOCUS CHI-SQUARE TEST FOR HETEROGENEITY AMONG POST LARVAL SETTLEMENT STAGES

| LOCUS | $X^2$-VALUE | d.o.f. | PROBABILITY |
|---|---|---|---|
| *LDH-1* | 3.48 | 3 | $P > 0.05$ |
| *PGM* | 10.36 | 6 | $P > 0.05$ |
| *PEP-B1* | 269.05 | 3 | $P < 0.001$ |
| *PEP-B2* | 150.58 | 6 | $P < 0.001$ |
| *PEP-D* | 3.65 | 6 | $P > 0.05$ |
| *GPI* | 4.37 | 6 | $P > 0.05$ |
| SUM | 441.50 | 30 | $P < 0.001$ |

TABLE 9

ALLELE FREQUENCIES FOR POST-LARVAL SAMPLES; 1990 AND 1991
N = Number of individuals

| | | 1990 | | | | | | | 1991 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOCUS | ALLELE | MAY | JUNE | JULY | AUG. | SEPT. | OCT. | NOV. & DEC. | JAN,FEB & MAR. | APR. | MAY & JUNE | JUL. | AUG. & SEPT. |
| *LDH-1* | N | - | - | - | - | - | - | 19 | 94 | 97 | 89 | 51 | 85 |
| | *100* | - | - | - | - | - | - | 1.000 | 1.000 | 1.000 | 0.994 | 1.000 | 0.994 |
| | *110* | - | - | - | - | - | - | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.006 |
| *PGM* | N | 100 | 92 | 100 | 99 | 90 | 72 | 58 | 126 | 96 | 88 | 64 | 109 |
| | *100* | 0.970 | 0.984 | 0.980 | 0.965 | 0.961 | 0.958 | 0.974 | 0.976 | 0.974 | 0.989 | 0.977 | 0.986 |
| | *200* | 0.030 | 0.016 | 0.015 | 0.030 | 0.033 | 0.035 | 0.017 | 0.012 | 0.021 | 0.011 | 0.023 | 0.009 |
| | *-200* | 0.000 | 0.000 | 0.005 | 0.005 | 0.006 | 0.007 | 0.009 | 0.012 | 0.005 | 0.000 | 0.000 | 0.005 |
| *PEP-D* | N | - | - | - | 66 | 45 | 49 | 26 | 91 | 63 | 34 | 32 | 51 |
| | *100* | - | - | - | 0.652 | 0.633 | 0.521 | 0.481 | 0.478 | 0.611 | 0.500 | 0.531 | 0.539 |
| | *95* | - | - | - | 0.326 | 0.322 | 0.469 | 0.500 | 0.478 | 0.373 | 0.500 | 0.453 | 0.451 |
| | *105* | - | - | - | 0.022 | 0.045 | 0.010 | 0.019 | 0.044 | 0.016 | 0.000 | 0.016 | 0.010 |
| *GPI* | N | 100 | 90 | 100 | 99 | 90 | 79 | 58 | 123 | 98 | 97 | 64 | 109 |
| | *100* | 0.995 | 0.994 | 1.000 | 0.990 | 0.983 | 0.987 | 0.991 | 0.992 | 1.000 | 1.000 | 0.984 | 0.990 |
| | *60* | 0.005 | 0.006 | 0.000 | 0.010 | 0.011 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *120* | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.013 | 0.009 | 0.004 | 0.000 | 0.000 | 0.000 | 0.005 |
| | *80* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.016 | 0.005 |

TABLE 10

SUMMARY OF PAIRWISE COMPARISONS BETWEEN POST-LARVAL SAMPLES AND ADULT BERMUDA POPULATION.

Significance tests are total $X^2$-values, produced by summing individual locus by locus comparisons (number of tests shown in brackets).
N.S. = not significant

| 1990 | | 1991 | |
|---|---|---|---|
| MAY | N.S. (2) | JAN., FEB. & MAR. | N.S. (4) |
| JUNE | N.S. (2) | APR. | N.S. (3) |
| JULY | N.S. (2) | MAY & JUNE | $P < 0.05$ (3) |
| AUG. | N.S. (3) | JULY | N.S. (4) |
| SEP. | N.S. (3) | AUG. & SEP. | N.S. (4) |
| OCT. | $P < 0.05$ (3) | | |
| NOV. & DEC. | N.S. (4) | | |

TABLE 11

DETAILS OF PLANKTON TOWS

Tows conducted on F/V Protector except * = R/V Calamus

All Phyllosoma are *Panulirus* species unless stated.
S = small, M = mid, L = large

| DATE | MOON PHASE | PLANKTON TOWS | PHYLLOSOMA |
|---|---|---|---|
| 21/22-6-90* | New Moon | 1) 70 Miles N. Bda. | 1 L (*Scyllaridae*) |
| | | 2) 60 Miles N. Bda. | - |
| | | 3) 50 Miles N. Bda. | 4 L (*Scyllaridae*) |
| 5/6-7-90 | 1st Qtr + 5 Days | 1) 13 Miles NNE. Bda. | 1 M |
| | | 2) 10 Miles NNE. Bda. | 1 M |
| | | 3) 8 Miles NNE. Bda. | 39 S |
| | | 4) 5 Miles ENE. Bda. | 50 S |
| 30/31-8-90 | 1st Qtr + 2 Days | 1) 8 Miles ESE. Bda. | 2 M |
| | | 2) 7 Miles ESE. Bda. | 3 M, 3 S |
| | | 3) 6 Miles ESE. Bda. | 1 M, 1 S |
| | | 4) 3 Miles ESE. Bda. | 1 M, 108 S |
| | | 5) 3 Miles ESE. Bda. | 3 M, 100 S |
| 28/29-11-90 | 1st Qtr + 3 Days | 1) 2 Miles SE. Bda. | - |
| | | 2) 3 Miles SE. Bda. | 3 M |
| | | 3) 5 Miles SE. Bda. | 2 M |
| | | 4) 6 Miles SE. Bda. | - |
| 16/17-1-91 | New Moon | 1) 7 Miles SE. Bda. | - |
| | | 2) 5 Miles SE. Bda. | - |
| | | 3) 5 Miles SE. Bda. | - |
| | | 4) 3 Miles E. Bda. | 1 L (*Scyllaridae*) |
| | | 5) 2 Miles E. Bda. | - |
| 16/17-4-91 | New Moon + 2 Days | 1) 7 Miles E. Bda. | 2 L, 3 M |
| | | 2) 6 Miles E. Bda. | 1 L, 2 M |
| | | 3) 5 Miles E. Bda. | 2 M |
| | | 4) 3 Miles E. Bda. | - |
| | | 5) 2 Miles E. Bda. | - |
| 12/13-6-91 | New Moon | 1) 6 Miles E. Bda. | - |
| | | 2) 5 Miles E. Bda. | 10 S |
| | | 3) 4 Miles E. Bda. | 18 S |
| | | 4) 2 Miles E. Bda. | 1 S |
| | | 5) 1 Mile E. Bda. | 4 S |
| 12/13-8-91 | New Moon + 2 Days | 1) 4 Miles SSE. Bda. | 1 M |
| | | 2) 3 Miles SSE. Bda. | - |
| | | 3) 2 Miles SSE. Bda. | - |
| | | 4) 1 Mile SE. Bda. | 65 S |
| | | 5) 1 Mile ESE. Bda. | 115 S |
| 10/11-9-91 | New Moon | 1 surface) 3 Miles SSE. Bda. | 36 M, 1 S, 1 M (*Scyllaridae*) |
| | | 1 downrig) | 3 L, 73 M, 1 M (*Scyllaridae*) |
| | | 2 surface) 3 Miles SSE. Bda. | 3 L, 20 M |
| | | 2 downrig) | 2 L, 47 M |
| | | 3 surface) 1 Mile SSE. Bda. | 1 M, 1000+ S |
| | | 3 downrig) | 1 M, 1000+ S |
| | | 4 surface) 1 Mile N. Bda. | 1000+ S |
| | | 4 downrig) | 1000+ S |

TABLE 12

ALLELE FREQUENCIES FOR THREE SPECIES OF PHYLLOSOMA; *P. ARGUS, P. GUTTATUS* AND A MEMBER OF THE FAMILY *SCYLLARIDAE*.

Allele produces are numbered by mobility relative to the most common *P. argus* allele.

| LOCUS | ALLELE | PANULIRUS ARGUS | PANULIRUS GUTTATUS | FAMILY SCYLLARIDAE |
|---|---|---|---|---|
| *GPI* | N | 44 | 23 | 1 |
| | *100* | 0.989 | 0.022 | 0.000 |
| | *120* | 0.011 | 0.978 | 0.000 |
| | *108* | 0.000 | 0.000 | 1.000 |
| *MDH-1* | N | 43 | 21 | - |
| | *100* | 1.000 | 0.000 | - |
| | *130* | 0.000 | 0.905 | - |
| | *70* | 0.000 | 0.095 | - |
| *MDH-2* | N | 43 | - | - |
| | *100* | 1.000 | - | - |
| *PEP-B2* | N | 45 | 20 | - |
| | *95* | 1.000 | 1.000 | - |
| *PEP-B3* | N | - | 19 | - |
| | *80* | - | 1.000 | - |

FIGURE 1. SAMPLING SITES FOR BERMUDA ANIMALS

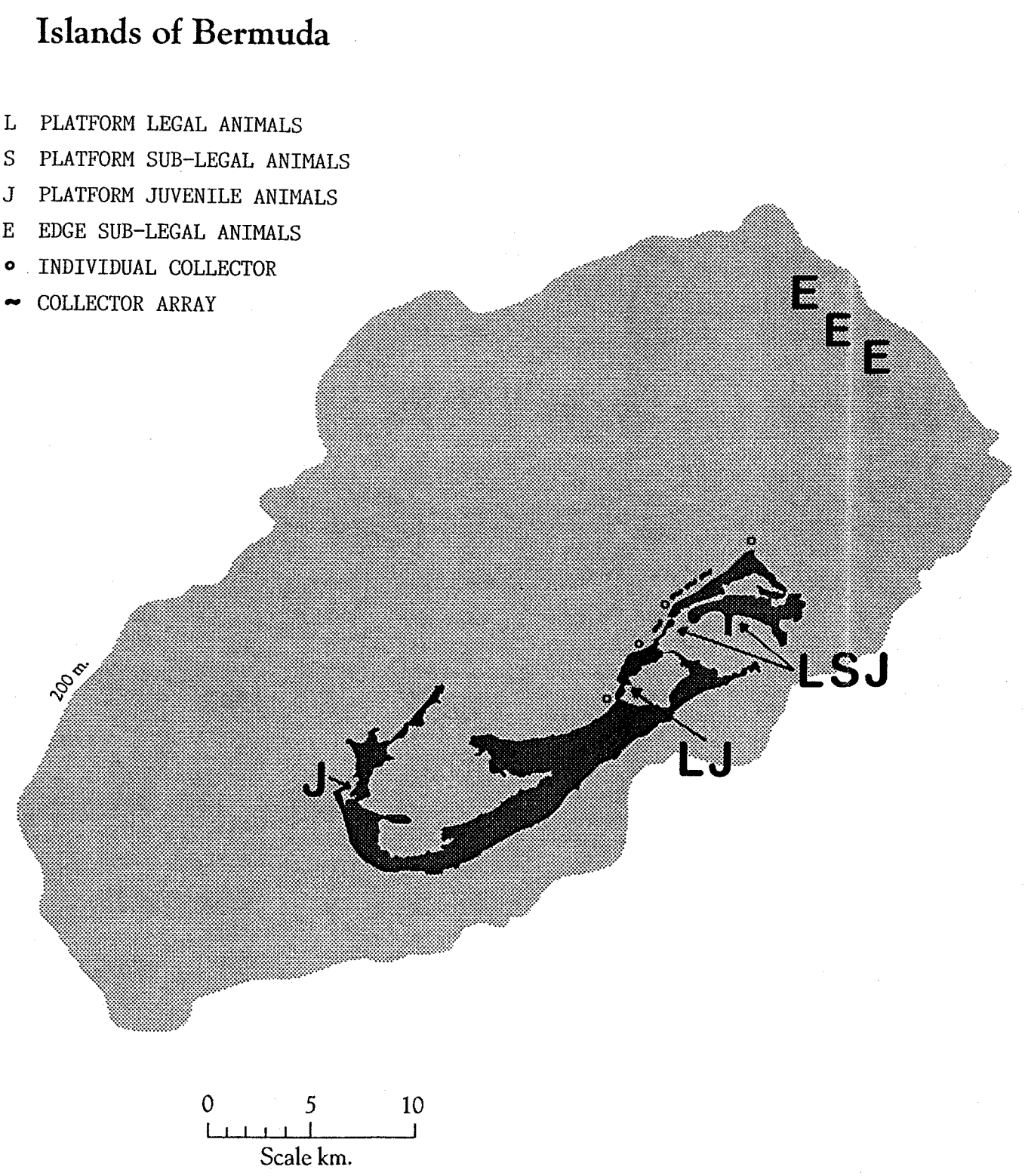
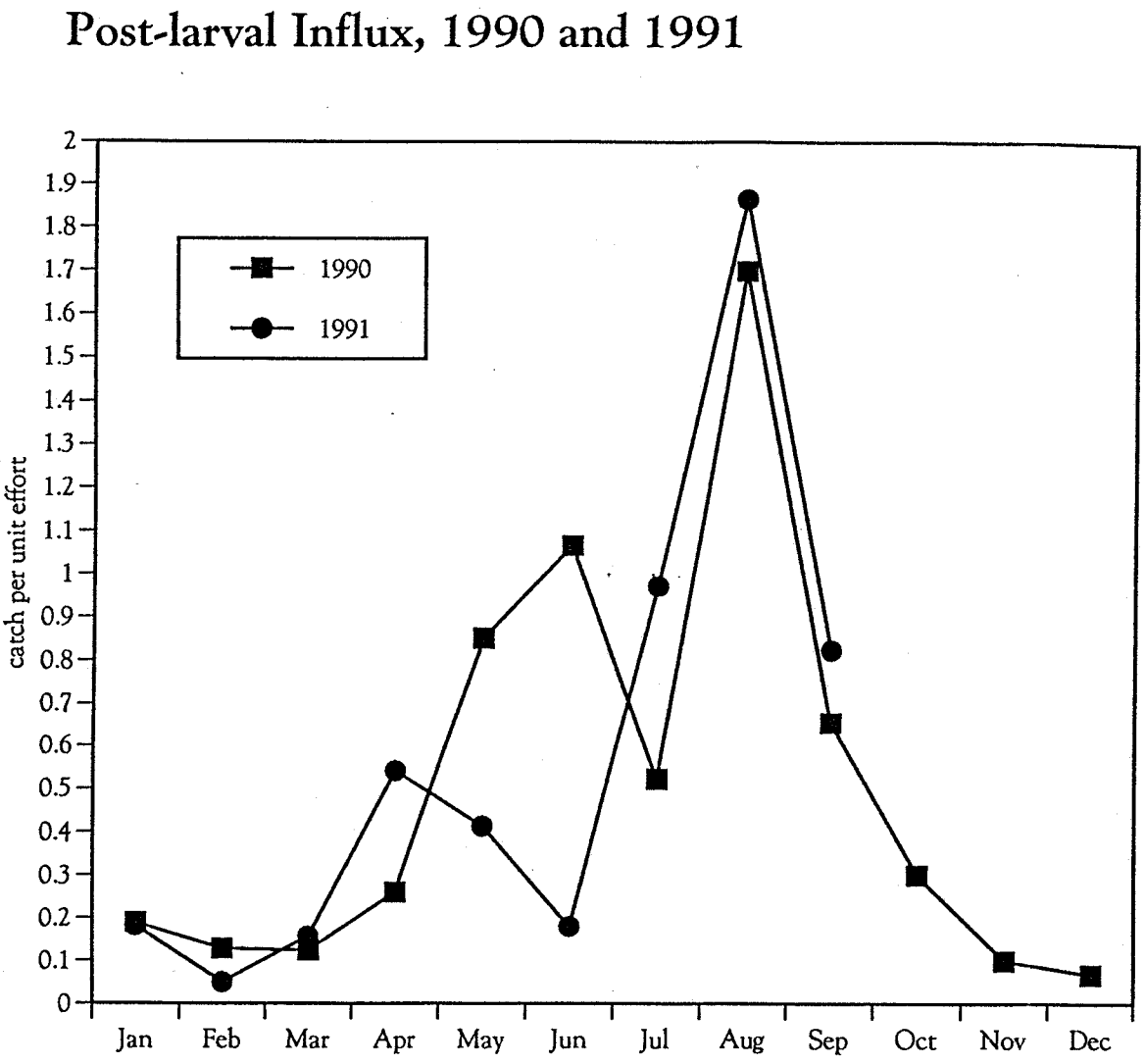


FIGURE 2. SAMPLING SITES FOR CARIBBEAN ANIMALS

| | | |
|---|---|---|
| B BERMUDA | E ELLIOTT KEY * | V VIRGIN ISLANDS * |
| M MIAMI | K KEY WEST * | J JAMAICA * |
| F FLORIDA KEYS | C CANCUN * | T TRINIDAD * |

* FROM MENZIES (1981)

FIGURE 3. POST-LARVAL SETTLEMENT BY MONTH



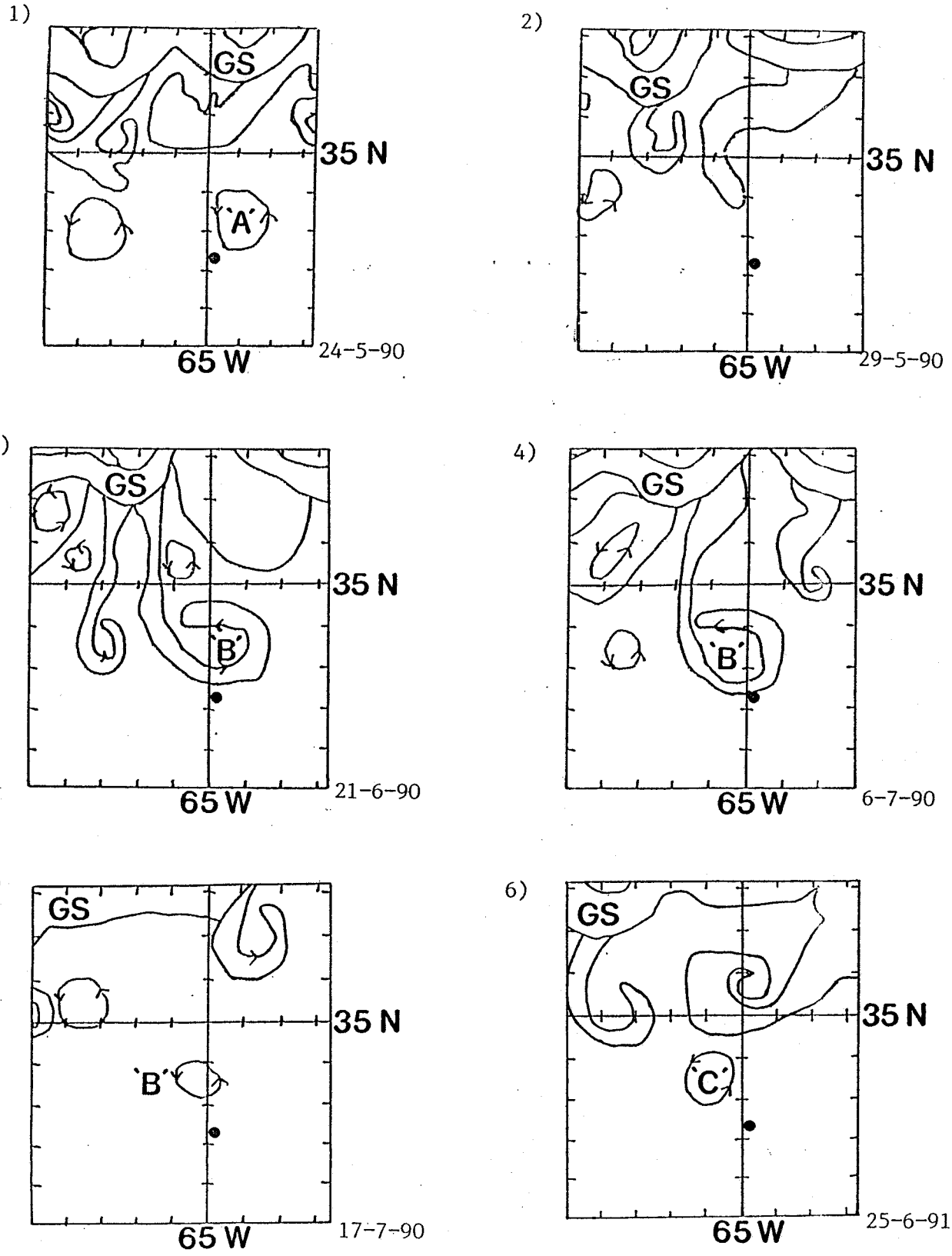



FIGURE 4 EDDY ACTIVITY IN WATERS SURROUNDING BERMUDA, MAY 1990 - JUNE 1991

MAPS WERE REDRAWN FROM NOAA STELLITE CHARTS FOR SEA SURFACE TEMPERATURES
GS = GULF STREAM, CIRCLE = BERMUDA, 'A', 'B' AND 'C' = EDDIES.