S. Afr. J. mar. Sci. 21: 271–277
1999

ALLOZYME FREQUENCIES INDICATE LITTLE GEOGRAPHIC VARIATION AMONG STOCKS OF GIANT TIGER PRAWN *PENAEUS MONODON* IN THE SOUTH-WEST INDIAN OCEAN

A. T. FORBES, N. T. DEMETRIADES*, J. A. H. BENZIE† and E. BALLMENT†*

The giant tiger prawn *Penaeus monodon* is an important component of prawn fisheries in the south-west Indian Ocean and the species of choice in prawn mariculture over much of the Indo-Pacific. Allozyme analysis of specimens collected between December 1996 and June 1997 from the Thukela Banks off KwaZulu-Natal, South Africa, the Sofala Banks off the Zambesi River in Moçambique and the north-western coast of Madagascar showed that, despite geographic separations of up to 2 000 km, Nei's unbiased genetic distance estimates between populations did not exceed 0.002. Mean heterozygosities within populations varied between 0.08 and 0.12 and allele frequencies were generally consistent with expectations under conditions of Hardy-Weinberg equilibrium. F_{st} values of 0.007 over all five populations sampled were not significant and indicate panmixis with high gene flow among all populations.

The giant tiger prawn *Penaeus monodon* Fabricius has a range extending from the east coast of South Africa to China and Australia (Grey *et al.* 1983). Throughout this area, it is a valued fisheries target and is being cultured over a steadily increasing proportion of the range. In recent years, this has included Moçambique, Madagascar and South Africa. Broodstock for the southern African operations is generally obtained locally, but these stocks are being used increasingly for operations farther afield.

The major prawn grounds in the South-West Indian Ocean are found on the Thukela Banks off KwaZulu-Natal in South Africa, Maputo Bay in southern Moçambique, the Sofala Banks off the Zambesi River and several grounds off the western and north-western coasts of Madagascar (Fig. 1). These are localized on shallow areas of the continental shelf, typically in conjunction with suitable estuarine nursery grounds. This results in a disjunct distribution of prawn habitats, particularly along the African coast, where they are typically separated by inshore, deep-water areas. The Madagascar grounds are separated from the African coast by the 2 000–3 000 m deep and 450–500 km wide Moçambique Channel. Despite this geographic separation, the species composition and proportions in the commercial catches in all the areas are similar (Ulltang *et al*. 1980, Demetriades and Forbes 1993, Laroche *et al.* 1995), with *P. monodon* generally contributing 5–10% of the total catch.

The relationships between the Moçambique, Madagascar and South African populations of any of the prawn species involved are unknown. It was suggested by Forbes and Cyrus (1991), on the basis of postlarval studies, that recruitment into the St Lucia Lake system and Richards Bay nursery grounds in South Africa derives primarily from local Thukela Bank stocks rather than from populations farther north. A knowledge of the relationships between the South-West Indian Ocean stocks, all of which are exploited or used as broodstock sources for mariculture, is critical for the long-term management of the resource. This paper describes the first comparison of allozymes from populations of *P. monodon* from central Moçambique, the north-western coast of Madagascar and from St Lucia and Richards Bay in KwaZulu-Natal, South Africa, in an attempt to resolve the degree of affinity of these stocks.

MATERIAL AND METHODS

Collection of samples

Specimens of *P. monodon* were obtained pre-frozen from commercial fisheries operating on the Sofala Banks off the Zambesi River (20°00´S, 35°E) in Moçambique and off Nosy Bé (13°15´S, 48°15´E) in north-western Madagascar. Specimens from Mahajanga (15°30´S, 46°30´E) south of Nosy Bé were obtained fresh from commercial trawlers. Specimens from St Lucia (28°30´S, 32°30´E) and Richards Bay (28°45´S,

* School of Life and Environmental Sciences, University of Natal, Durban, South Africa, 4041. E-mail: forbesa@biology.und.ac.za
† Australian Institute of Marine Science, P.M.B 3, Townsville, M.C. Queensland 4810, Australia. E-mail: jbenzie@aims.gov.au

Manuscript received: August 1998

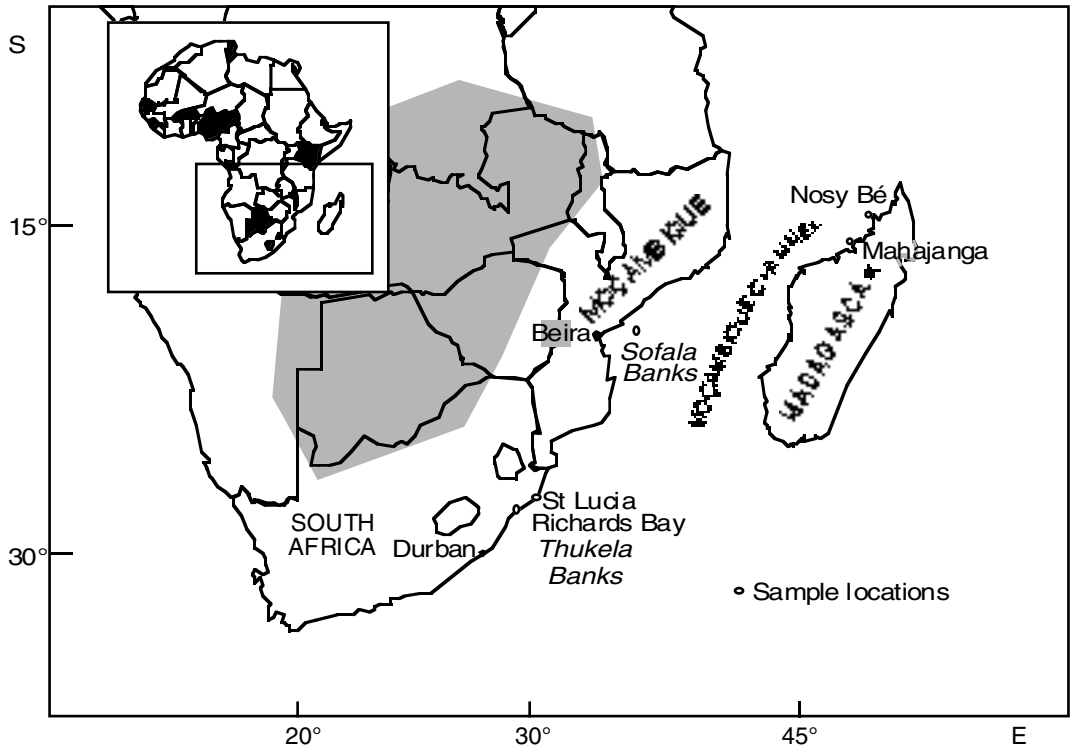

Fig. 1: Collection sites of *P. monodon* in the South-West Indian Ocean

32´E) in KwaZulu-Natal were collected using a beam trawl and immediately frozen on dry ice. In all cases, processing involved removal of the basal pleopod joints with the enclosed muscle, as well as a sample of abdominal muscle in the case of smaller individuals. Samples were preserved in cryotubes at –75°C or in liquid nitrogen before analysis.

Laboratory analysis

Portions of tissue were chipped from the frozen sample and homogenized, using a stainless steel pestle, in an approximately equal volume of grinding buffer (100 mM Tris-HCl, pH 8) in the well of a porcelain cavity tile. Proteins were separated electrophoretically, either by applying the sample directly to cellulose acetate gels or by absorbing the sample into chromatography paper wicks (7 mm × 3 mm, Whatman No. 3) and applying these to horizontal, 13% starch gels.

Seven proteins that were shown to be polymorphic in previous studies of genetic variation in *P. monodon* (Benzie *et al.* 1992) were assayed: glucose-6-phosphate isomerase (GPI) – E.C.5.3.1.9; peptidase (leu-gly-gly substrate) (LGG) – E.C. 3.4.11/13; peptidase (leu-tyr substrate) (LT) – E.C. 3.4.11/13; malate dehydrogenase (MDH) – E.C. 1.1.1.37; mannose-6-phosphate dehydrogenase (MPI) – E.C. 5.3.1.8; phosphogluconate dehydrogenase (PGD) – E.C. 1.1.1.44 and phosphoglucomutase (PGM) – E.C. 5.4.2.2. Starch and cellulose acetate gel recipes, buffers and running conditions for all the above enzymes followed Benzie *et al.* (1992), with the exception of MPI, LGG and LT. Tests with tris-glycine buffer (TG) had shown better resolution for these three systems and these were run at 500 V for 5 hours with 25 mM T-G, pH 8.4, as both electrode and gel buffer (Scandalios 1969). In some cases, PGM was run on TG instead of a tris-citrate buffer (TC7), because the banding pattern was the same on either buffer. During the analysis, PGD was found to be invariant in these animals and was omitted from further analysis.

All enzymes were visualized by using standard stain recipes (Shaw and Prasad 1970, Richardson *et al.*

Table I: Gene frequencies in five populations of *P. monodon* from southern Africa and Madagascar

Locus	Gene frequency				
	St Lucia	Richards Bay	Sofala Banks	Mahajanga	Nosy Bé
GPI					
123	0.012	0	0.005	0.013	0
100	0.922	0.969	0.947	0.962	0.977
83	0.066	0.031	0.037	0.013	0.023
77	0	0	0.011	0.013	0
LGG					
104	0	0.006	0	0.013	0
100	0.994	0.969	0.995	0.988	1.000
95	0.006	0.025	0.005	0	0
LT-1					
100	0.873	0.957	0.937	0.850	0.807
95	0.127	0.043	0.063	0.125	0.182
LT-2					
104	0.006	0.019	0.068	0.025	0
100	0.994	0.907	0.932	0.950	0.955
96	0	0.074	0	0.025	0.045
MDH-1					
111	0	0	0	0	0.023
100	1.000	1.000	1.000	1.000	0.977
MDH-2					
116	0	0.006	0.016	0.025	0
100	1.000	0.994	0.984	0.962	1.000
89	0	0	0	0.013	0
MPI					
105	0.036	0.012	0.047	0.038	0.023
100	0.542	0.556	0.432	0.587	0.534
95	0.343	0.327	0.384	0.275	0.375
91	0.078	0.105	0.137	0.100	0.068
PGM					
105	0.012	0	0	0	0
100	0.988	0.994	0.984	0.988	1.000
85	0	0	0	0.013	0
74	0	0.006	0.016	0	0
n	83	81	95	40	44

n = Number of individuals screened

1986) and alleles were labelled according to their migration distance relative to that of the most common allele, which was assigned a value of 100.

Statistical analyses

Calculations of basic genetic parameters, tests for conformity to Hardy-Weinberg equilibrium frequencies, genetic distance and cluster analyses were carried out using the BIOSYS-1 package (Swofford and Seelander 1981). It should be noted that, although the use of only polymorphic loci will both inflate genetic distance and bias heterozygosity estimates relative to those obtained using a random sample of loci, analysis of patterns of population differentiation will not be affected. Tests of conformance of allele frequencies to Hardy-Weinberg equilibrium (Elston and Forthofer 1977) and tests of pairwise comparisons of genotype frequencies between populations used significance values appropriately corrected for multiple simultaneous tests following Miller (1966).

F-statistics were calculated following the method of Weir and Cockerham (1984), which explicitly takes into account differences in sample sizes among the populations tested. The statistical significance of F_{st} was calculated using the equation given in Waples (1987) to estimate χ^2. F_{st} was used to calculate the average number of migrants per generation (N_em), in which

$$N_em = [(1/F_{st}) - 1]/4 \quad .$$

RESULTS

In each of the eight polymorphic loci screened, one allele was dominant in all five populations, with the other alleles occurring at a low frequency (Table I, Fig. 2). The five populations had mean heterozygosities ranging from 0.08 to 0.012 and a mean number of alleles per locus ranging from 1.8 to 2.4 (Table II). Gene frequencies at seven of the polymorphic loci (Table III) showed no significant deviation from Hardy-Weinberg expectations, with one exception. LT-1 showed significant heterozygote deficits, with several heterozygotes fewer than expected occurring in the St Lucia, Sofala Banks and Mahajanga populations. Mean F_{is} values, and those for most loci, were not significant, indicating no strong within-population structuring (Table IV). Two individual loci showed significant F_{is} values; LT-1, reflecting the deficits of heterozygotes detected in the chi-square tests, and MDH-1, due to the strong statistical but biologically unimportant effect of the occurrence of one homozygote (MDH-1^{111} – Table I) in the absence of any heterozygote for that locus.

Although the F_{st} value for LT-2 was significant, the lack of significance at other loci and of the mean, indicated no genetic differentiation between populations (Table IV). Pairwise comparisons showed no further structure, in that none of the pairwise values was significantly different from zero (Table V). In keeping with this pattern, estimates of N_em suggest that much mixing between populations occurs, with an average N_em of 35.5. A dendrogram (Fig. 3) constructed from Nei's genetic distances between all populations (Table VI) confirmed the lack of structure revealed by the *F*- statistics.

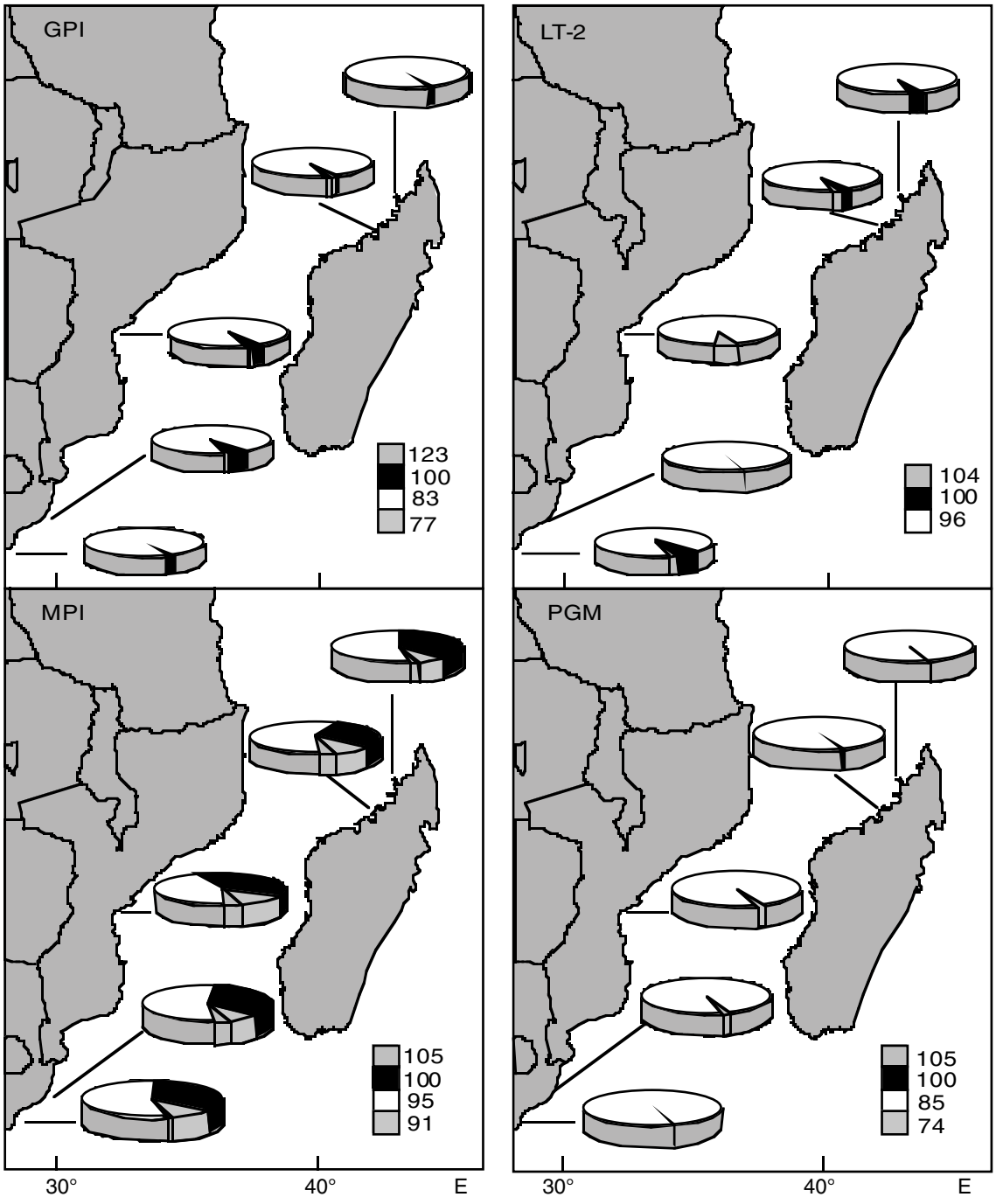

Fig. 2: Pie charts illustrating the variation in gene frequencies at GPI, LT-2, MPI and PGM in South-West Indian Ocean *P. monodon* populations

Table II: Genetic variability measures (with standard errors where appropriate) for five populations of *P. monodon* from southern Africa and Madagascar. A locus was considered polymorphic if more than one allele was detected; expected heterozygosity: heterozygosity expected under conditions of Hardy-Weinberg equilibrium (Nei's unbiased estimate, Nei 1978)

Population	Mean sample size/locus	Mean number alleles/locus	% loci polymorphic	Mean heterozygosity	
				Direct count	Expected count
St Lucia	83 (0.0)	2.0 (0.3)	66.7	0.102 (0.070)	0.111 (0.065)
Richards Bay	81 (0.0)	2.2 (0.3)	77.8	0.102 (0.053)	0.111 (0.061)
Sofala Banks	95 (0.0)	2.2 (0.3)	77.8	0.115 (0.074)	0.119 (0.068)
Mahajanga	40 (0.0)	2.4 (0.4)	77.8	0.092 (0.055)	0.119 (0.061)
Nosy Bé	44 (0.0)	1.8 (0.3)	55.6	0.083 (0.057)	0.112 (0.064)

DISCUSSION

It is clear from the results obtained from samples of Madagascar, Moçambique and South African populations that little or no genetic structure exists at the allozyme level which might allow the recognition of separate stocks of *P. monodon* in those regions of the South-West Indian Ocean. To some extent, this accords with the statement by Dall *et al.* (1990) that genetic diversity in the Penaeidae is among the lowest recorded for any animals. The present study did, however, show that diversity within these populations was comparable with that found in a small number of *P. monodon* sampled from a single Australian site (Mulley and Latter 1980) and with populations from Fiji, the Philippines, Taiwan and cultured stocks from Tahiti (Lioe 1984).

It also appears that, under suitable conditions, it is possible for genetic differences between populations of *P. monodon* to arise. Highly significant genetic differences have been found between six populations of *P.monodon* from sites in northern and north-eastern Australia and a western Australian population (Benzie *et al.* 1992). The differences in this case appear to be associated with changes in sea levels over the last 20 000 years, which resulted in the closure of the Torres Strait between Australia and New Guinea from about 18 000 BP until 8 000 BP. The genetic data indicate a colonization of the areas to the west of the Torres Strait from the east after restoration of the marine link. The low diversity of the western Australian population and the fact that all genotypes in the west were found in the northern and eastern populations suggest that the western population was derived from a small founder population from the

Table III: Probabilities that genotype frequencies observed in the five populations of *P. monodon* conform to expectations under conditions of Hardy-Weinberg equilibrium using the exact test (Elston and Forthofer 1977)

Locus	St Lucia	Richards Bay	Sofala Bank	Mahajanga	Nosy Bé
GPI	0.068	1.000	1.000	1.000	1.000
LGG	1.000	1.000	1.000	1.000	–
LT-1	0*	0.209	0*	0*	0.214
LT-2	1.000	1.000	1.000	0.075	0
MDH-1	–	–	–	–	0.011
MDH-2	–	1.000	1.000	1.000	–
MPI	0.185	0.074	0.023	0.514	0.377
PGM	1.000	1.000	1.000	1.000	–

* $p < 0.05$

Table IV: Summary of *F*-statistics (Weir and Cockerham 1984)

Locus	F_{st}	F_{is}	F_{it}
GPI	0.001	0.022	0.022
LGG	0.006	0.016	0.009
LT-1	0.006	0.487 ***	0.490
LT-2	0.017 **	0.101	0.117
MDH-1	0.011	1.000***	1.000
MDH-2	0.010	0.017	0.007
MPI	0.006	0.000	0.006
PGM	0.004	0.006	0.010
Mean	0.007	0.197	0.201

F_{is} = Standardized genetic variance within populations
F_{st} = Standardized genetic variance between populations
F_{it} = Total genetic variance
** $p > 0.01$
*** $p > 0.001$

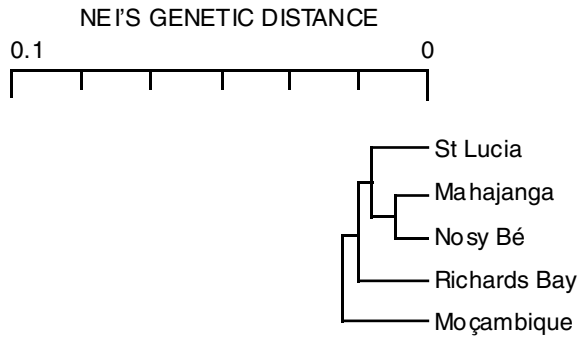

Fig. 3: Dendrogram showing the genetic relationships among South-West Indian Ocean populations of *P. monodon,* derived by using Nei's unbiased *D* as the metric and the UPGMA clustering method. The cophenetic correlation for the tree was 0.95

north. The mechanism by which the uniqueness of the western Australian population is currently maintained is uncertain, because there are no obvious physical barriers to the dispersal of planktonic eggs and larvae, which is a feature typical of inshore penaeids.

A disruption of marine links akin to that associated with the above-mentioned New Guinea land bridge would not have been possible in the southern African/Madagascan region because of the coastal topography, but a sea level fall of some 135 m would have drastically reduced the availability of suitable shelf habitat worldwide (Dall *et al*. 1990) and would have been particularly significant in the south western Indian Ocean where the shelf is relatively narrow. Subsequent recolonization of any defaunated areas would have been from tropical areas to the north after sea level recovery and any subsequent genetic divergence between populations in this region would depend on time and geographic separation.

The maximum distance between stocks sampled in this study was c. 2 000 km. The absence of any genetic distinctiveness suggests that, despite this geographical separation, there is sufficient genetic exchange between the different populations to prevent any differentiation. It is possible that exchange is brought about by adults emigrating from juvenile estuarine nursery grounds, but it is more likely that planktonic eggs and larvae would be aided in their southward dispersal by the Moçambique and Agulhas currents. These would, however, be long-term effects, the importance of which may be measured by the lack of divergence in the populations of the region. The significance of dispersal of planktonic eggs and larvae and the predominantly south-flowing currents as factors influencing the short-term dynamics of the different populations remains to be resolved.

Table V: Summary of F_{st} pairwise among five populations of *P. monodon* from southern Africa and Madagascar (below the diagonal) and values of N_em (above the diagonal)

Population	1	2	3	4	5
1 St Lucia		20.6	31.0	35.5	49.8
2 Richards Bay	0.012		124.8	8	35.5
3 Moçambique	0.008	0.002		249.8	31.0
4 Mahajanga	0.007	0.000	0.001		∞
5 Nosy Bé	0.005	0.007	0.008	0.000	

Table VI: Genetic distances between five populations of *P. monodon* from southern Africa and Madagascar. Nei's (1978) unbiased genetic distance is below the diagonal and Rogers' (1972) genetic distance is above the diagonal

Population	1	2	3	4	5
1 St Lucia		0.029	0.031	0.024	0.020
2 Richards Bay	0.001		0.026	0.025	0.028
3 Moçambique	0.002	0.001		0.032	0.035
4 Mahajanga	0.000	0.000	0.002		0.027
5 Nosy Bé	0.000	0.001	0.001	0.000	

ACKNOWLEDGEMENTS

This work was carried out with the financial assistance of the South African Foundation for Research Development, the University of Natal, Durban, and the Australian Institute of Marine Science. We thank Mr R. H. Taylor and Ms C. E. Fox of the KwaZulu-Natal Nature Conservation Services and Mr A. Stamatis of Adcan Marine, KwaZulu-Natal, for assistance with obtaining samples from St Lucia and Richards Bay respectively. Dr V. G. Cockcroft (Centre for Dolphin Studies, Plettenberg Bay) made it possible to collect samples from Madagascar. Mr P. Vorwerk and Ms E. Lindenberg (formerly Biology Department, University of Natal) are acknowledged for assistance in the field. The experiments carried out comply with South African and Australian legal requirements. This paper is contribution number 951 from the Australian Institute of Marine Science.

LITERATURE CITED

BENZIE, J. A. H., FRUSHER, S. and E. BALLMENT 1992 — Geographical variation in allozyme frequencies of *Penaeus*

monodon (Crustacea: Decapoda) populations in Australia. *Aust. J. mar. Freshwat. Res.* **43**: 715–725.

DALL, W., HILL, B. J., ROTHLISBERG, P. C. and D. J. STAPLES (Eds) 1990 — The biology of the Penaeidae. *Adv. mar. Biol.* **27**: 489 pp.

DEMETRIADES, N. T. and A. T. FORBES 1993 — Seasonal changes in the species composition of penaeid prawns on the Tugela Bank, Natal, South Africa. *S. Afr. J. mar. Sci*. **13**: 317–322.

ELSTON, R. C. and R. FORTHOFER 1977 — Testing of Hardy-Weinberg equilibrium in small samples. *Biometrics* **33**: 536–542.

FORBES, A. T. and D. P. CYRUS 1991 — Recruitment and origin of penaeid prawn postlarvae in two South-East African estuaries. *Estuar. coast. Shelf Sci*. **33**(3): 281–289.

GREY, D. L., DALL, W. and A. BAKER 1983 — *A Guide to the Australian Penaeid Prawns*. Darwin; Northern Territory Government: 140 pp.

LAROCHE, J. BENOELY, E. and A. SIAVIRI 1995 — Biologie et pêche traditionelle des crevettes pènèides dans l'embouchere d'un fleuve en milieu semi-aride (sud-ouest de Madagascar). *Oceanologica Acta* **18**: 659–670.

LIOE, K. G. 1984 — La variabilite et la differentiation genetique de quelques especes de peneides. Ph.D. thesis, Universite des Sciences et Techniques du Languedoc, Montpellier: 72 pp.

MILLER, R. G. 1966 — *Simultaneous Statistical Inference*. New York; McGraw-Hill: 272 pp.

MULLEY, J. C. and B. D. H. LATTER 1980 — Genetic variation and evolutionary relationships within a group of thirteen species of penaeid prawns. *Evolution* **34**: 904–916.

NEI, M. 1978 — Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

ROGERS, J. S. 1972 — Measures of genetic similarity and genetic distance. *Univ. Tex. Publs* **7213**: 145–153.

RICHARDSON, B. J., BAVERSTOCK P. R. and M. ADAMS 1986 — *Allozyme Electrophoresis*. Sydney; Academic Press: 410 pp.

SCANDALIOS, J. G. 1969 — Genetic control of multiple forms of enzymes in plants: a review. *Biochem. Genet*. **3**: 37–79.

SHAW, C. R. and R. PRASAD 1970 — Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochem. Genet*. **4**: 297–320.

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. Heredity* **72**: 281–283.

ULLTANG, O., BRINCA, L. and C. SILVA 1980 — A preliminary assessment of the shallow water prawn stocks off Mozambique, north of Beira. *Revta Investnes. Pesq.*, *Mozambique* **1**: 1–69.

WAPLES, R. S. 1987 — A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* **41**(2): 385–400.

WEIR, B. S. and C. C. COCKERHAM 1984 — Estimating *F*-Statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.