CRC REEF RESEARCH TECHNICAL REPORT

GENETIC DETERMINATION OF SOURCES OF *ACANTHASTER PLANCI* RECRUITMENT

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The precise origins of outbreak populations of crown-of-thorns starfish (*Acanthaster planci*) are still unknown. Modelling studies, and inferences from known aspects of *A. planci* biology, suggest outbreaks first occur in the region between latitudes 14-16?S. Early genetic studies indicated that outbreak populations sampled from Townsville to the Swains region all came from one genetic source, presumably within this region.

The present study provides detailed information for the first time on the genetic structure of populations close to the beginning of an outbreak phase, sampling several populations from the presumed region of outbreak origin. The principal objective of the study was to determine sources of recruits to *Acanthaster planci* populations in an attempt to better define the nature of the origin of outbreak populations in the region of 14-16?S. This was to be achieved through interpretation of the genetic structure of those populations by specifically addressing the following questions: 1) Are recruits to those *A. planci* populations showing a recent increase in population size different in genetic composition, and hence derived from different sources? 2) Is the genetic composition of the present "outbreaks" the same as those described from the 1980s, implying both present and past outbreaks were derived from the same source area? 3) Are there differences in the genetic constitution of different age classes in the same population suggesting temporal variation in the source of recruits, and is this variation the same order of magnitude as any spatial variation observed?

Six populations of the crown-of-thorns starfish, *Acanthaster planci*, showing large increases in population size in 1994-95 were examined using 100-300 individuals collected from each population in Nov 1995 - Feb 1996. Nine allozyme loci used previously to determine population structure in the 1980s outbreak populations were analysed. The results showed: 1) no significant genetic differentiation among the recent outbreak populations for any age class (2-6 years old), consistent with the recruits to each population being derived from the same source in each year, 2) the 1996 samples clustered with the eight 1986 outbreak populations in a small part of the genetic space spanned by the 1986 non-outbreak populations, suggesting that the outbreak populations were derived from the same source in both 1986 and 1996 and, 3) no significant variation between age classes in each of the six populations suggesting that no change in the source of recruits to these reefs has occurred over the last 5-6 years.

The data showed no significant genetic differences between populations and indicated that all populations have derived their recruits from the same gene pool for the last five years. This does not necessarily mean that all populations arose from larvae from one source reef. In combination with data showing simultaneous increases in population size at several reefs (Engelhardt and Lassig (in press)) and hydrodynamic models indicating much of the region between 14?S and 16?S is highly connected, the genetic data indicate that *A. planci* found on several reefs in the region act as one panmictic population. The data are consistent with any one or more of a number of reef populations in this region contributing to the production of a pool of recruits that might build up over time until they contribute significantly to colonisation of reefs downstream and give rise to outbreaks in the Central and Southern GBR. The considerable similarity in the genetic constitution of the 1986 and 1996 sets of outbreak populations is consistent with both being derived from the same source area.

2. INTRODUCTION

Two major sets of outbreak populations of crown-of-thorns starfish have been recorded on the Great Barrier Reef (GBR), from 1966-1974 and from 1979-1991 (Moran 1986, Johnson 1992). A number of studies have suggested outbreak populations originate offshore from Cairns, although the exact spatial and temporal origin of the outbreaks remains unknown. Hydrodynamic models suggested that outbreaks begin inside the outer ribbon reefs between latitudes 14?30'S and 15?40'S (Dight et al. 1990a, 1990b) or from the simultaneous origin of several primary outbreaks in the region between 14?S and 16?S (Black 1992). Interpolating from analyses of the spread of outbreak populations north and south along the GBR, Moran et al. (1992) identified a likely epicentre for outbreaks close to latitude 16?S.

Genetic data from allozyme surveys of the second set of outbreak populations in 1986 supported the strong circumstantial evidence from ecological and oceanographic data that all the outbreak populations throughout the GBR were derived from the same source (Benzie and Stoddart 1992a). Inter-population differentiation in the outbreak populations was an order of magnitude less than those in the non-outbreak populations, outbreak populations were not significantly different in gene frequencies while non-outbreak populations were different and the outbreak populations clustered together in a small part of the genetic space spanned by the non-outbreak populations in an ordination analysis. Benzie and Stoddart (1988, 1992) argued that these data disproved the hypothesis that all outbreak populations were independent primary responses to the factor(s) inducing outbreak populations. In that situation, outbreak populations would be expected to have the same genetic structure as non-outbreak populations, and both outbreak and non-outbreak populations would be expected to spread equally through genetic space in ordination analyses.

The appearance of populations showing increases in the number of starfish in 1994 and 1995 (Engelhardt and Lassig (in press)) in or near the region Dight et al. (1990a, 1990b), Moran et al. (1992) and Black (1992) considered a possible epicentre for outbreaks suggested that another set of outbreaks had begun. Six of these populations were sampled with a view to determining their genetic constitution in order to infer sources of recruitment within the region 14-16?S and to identify markers that might allow detection of recruits derived from these populations in future outbreaks observed in the southern GBR. Specimens were aged using techniques developed for *Acanthaster planci* by Stump and Lucas (1990) and Stump (1994).

This permitted the determination of the genetic structure of specific age classes, and allowed a more detailed analysis of genetic structure among the populations in the region of primary outbreak, an opportunity not available to Benzie and Stoddart (1992).

3. MATERIALS AND METHODS

3.1 Field sampling

Samples of 103-298 individual starfish were obtained during Nov 1995 (Lizard Island only) or Feb 1996 from each of six reefs in the north central GBR on which *Acanthaster planci* had reached very high numbers. These were Lizard Island (14?40'S 145?28'E), Heldston Reef (14?57'S 145?29'E), Two Isles (15?01'S 145?27'E), Endeavour Reef (15?46'S 145?36'E), Pickersgill Reef (15?51'S 145?33'E), Evening (15?55'S 145?38'E) (Fig. 1). Animals were collected haphazardly from a single site covering several tens of metres until a minimum of 100 individuals had been obtained. At Lizard Island and Evening Reef, approximately 300 individuals were collected to ensure large sample sizes for different age classes were obtained from at least two sites.

Individual starfish were lifted from the substrate by divers and placed in seawater in a plastic bin. The animals were maintained alive until dissection on board the research vessel, usually within 6 hours of collection. A small incision was made at the base of an arm and the pyloric cecae extracted using forceps and placed in a plastic bag until approximately 5 ml of tissue had been collected. Air was excluded, the bag rolled in to a cylinder and placed immediately in liquid nitrogen. In the laboratory, frozen samples were broken into small chips and stored at -80°C until analysis. Diameters of the starfish were measured and samples of several spines obtained for later analysis of spine banding patterns. The age of each individual was determined using the results of spine banding and body size following Stump and Lucas (1990) and Stump (1994). Individuals classed as 1-year olds in the samples were the results of spawnings in the 1994/95 spawning season (Oct 94 - Feb 95), 2-year olds the result of spawnings in the 93/94 spawning season, and so on.

3.2 Laboratory analyses

Samples were ground in three drops of an aqueous solution of 0.04% ?-mercaptethanol in a ceramic well and a tissue square placed on the sample to act as a crude filter. Wicks of filter

paper were then soaked in sample solution for running on starch gels, and mapping pens used to load the sample on to Cellogel prior to electrophoresis (see Benzie 1990).

The seven polymorphic systems screened were malate dehydrogenase 1.1.1.37 (MDH), super oxidase dismutase 1.15.1.1 (SOD), glucose phosphate isomerase 5.3.1.9 (GPI), enolase 4.2.1.11 (ENOL), phophoglycerate kinase 2.7.2.3 (PGK), peptidase 3.4.13.9 on leucyl proline substrate (LP), and peptidase 3.4.11.* on leucyl tryosine substrate (LT). MDH, SOD and GPI were run on 12% starch gels using a TC7 buffer (electrode buffer 135 mM Tris, 43 mM citric acid to pH 7.0 using HCL; gel buffer 9.6 mM Tris, 3 mM citric acid, pH 7.0), ENOL, LP and LT were run on cellulose acetate gels (Cellogel; Milan) using 10 mM CP 6.4 buffer (10 mM disodium hydrogen phosphate, 2.5 mM citric acid) and PGK was run on cellulose acetate gels using 50 mM TM 7.8 buffer (50 mM Tris, 20 mM Maleic acid).

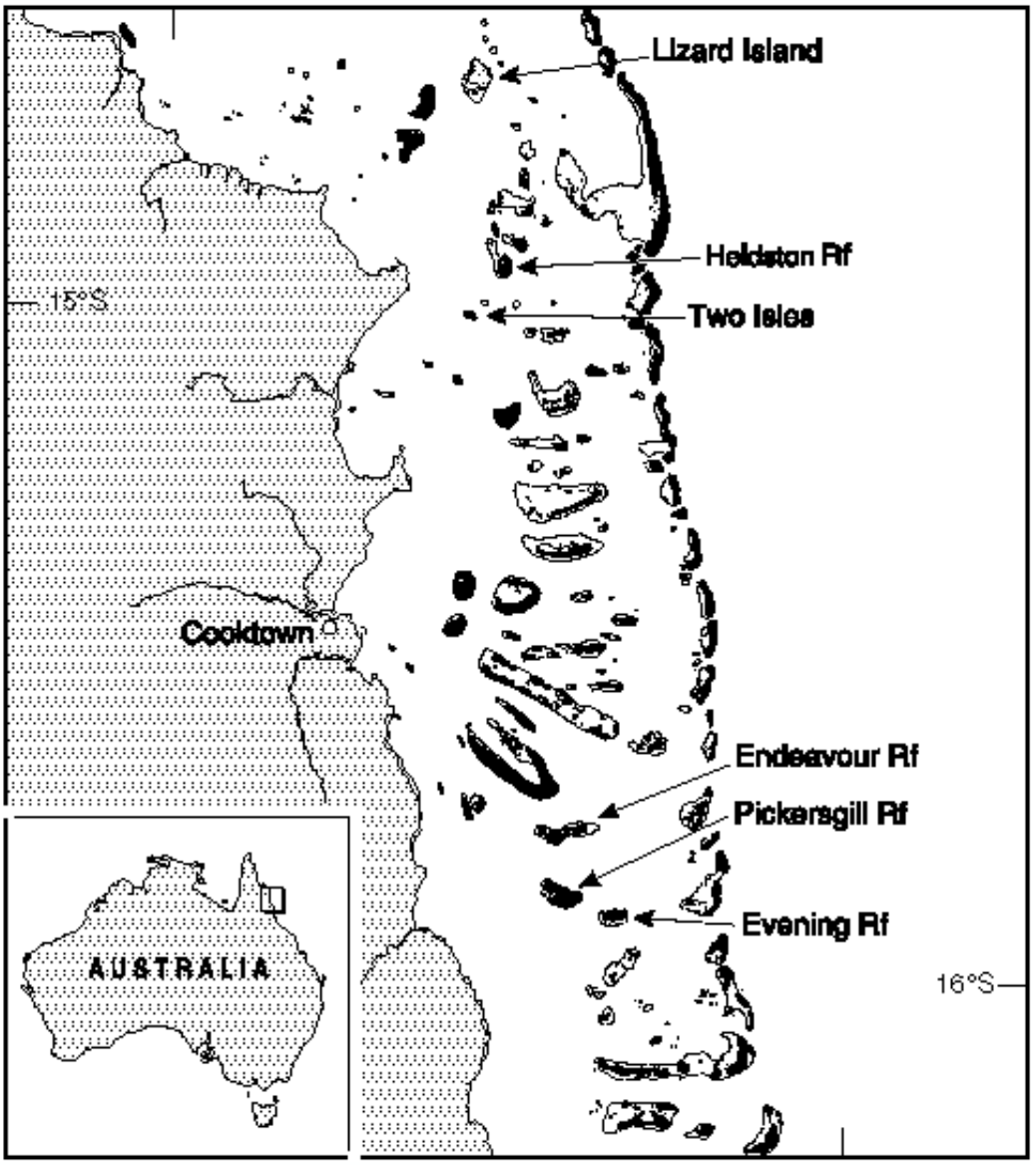

Figure 1. Location of samples sites on the Great Barrier Reef.

3.3 Statistical analyses

Gene frequencies, basic statistics of genetic variability, standard F_{IS} and F_{ST} estimates and clustering procedures were carried out using programs in the BIOSYS package (Swofford and Selander 1981). In all tests comparing gene frequencies between samples or testing conformation to Hardy-Weinberg expectations, the significance values used were appropriately corrected for multiple simultaneous tests (Miller 1966). Multidimensional scaling analyses were performed with a program in the PATN analysis package using the two-step algorithm (Belbin 1987).

F-statistics were used to partition genetic variation into that occurring within populations (F_{IS}) and that occurring between populations (F_{ST}) using equations which take account of differences in sample size between populations (Weir and Cockerham 1984). The significance of F_{IS} and F_{ST} values was tested using the chi-squared statistic. For tests of F_{IS}, chi-squared equals $N(F_{IS})^2$ (k-1) with degrees of freedom (d.f.) equal to (k(k-1))/2 where N is the total number of individuals sampled and k the number of alleles at the locus. For tests of F_{ST}, chi-squared equals $2N(F_{ST})$ (k-1) with d.f. = (k-1)(s-1), where N and k are as defined above and s is the number of populations sampled (Waples 1987). A number of cells had low expected values, but analyses pooling rare alleles did not alter the nature of the results. The average number of migrants exchanged between populations (N_em) was calculated as $N_em = ((1/F_{ST})-1)/4$. An estimate of the variance of the mean number of immigrants was obtained by jacknifing loci as described by Weir and Cockerham (1984), after Reynolds et al. (1983), to obtain 95% confidence limits for F_{ST} and then calculating N_em from these. The data for the 1995 outbreaks were compared with the data from the 1986 outbreak and non-outbreak populations reported in Benzie and Stoddart (1992).

4. RESULTS AND DISCUSSION

4.1 Genetic differentiation of populations and between age classes

Gene frequencies and statistics describing genetic variation for the six populations (pooling all age classes) and for each age class in each population are given in the Appendix (Tables A1-14). Gene frequencies were similar in all populations and age classes.

No significant differentiation between populations was detected by F_{ST} for any of the age classes sampled (Table 1). Although the mean value of F_{ST} rose an order of magnitude in 7-

year old animals, the sample sizes of individuals within populations and the number of populations for this age group were small, and the value was not statistically significant. Calculation of F_{ST} between different age classes within populations revealed no significant inter-generation structure (Table 2). F_{ST} values rose an order of magnitude in the comparison of 4- and 5-year olds in the Heldston Reef population alone, but the value was not significant.

The lack of significant differences between all six populations in every age class, and the lack of genetic differentiation between age classes within each population provides evidence that the 1996 outbreak populations received recruits from the same genetic source from 1989 - 1995. It could be argued that the allozyme data do not provide sufficient resolution to detect the gene frequency differences which exist between these populations. However, allozymes were sufficient to detect significant genetic differences between the 1986 non-outbreak populations, where sample sizes were on average about half those obtained for the outbreak populations.

4.2 Comparison of 1986 and 1996 outbreak population sets

When populations were mapped in multivariate genetic space using a multidimensional scaling analysis the non-outbreak populations spread over much of the plot while outbreak populations from both 1996 and 1986 were clustered close to each other (Fig 2). The area of the space occupied by both sets of outbreak populations was only 0.1 that occupied by non-outbreak populations. The general pattern was similar to PCA analysis of the 1986 data reported by Benzie and Stoddart (1992, Fig 3). Variants at a number of loci were significantly correlated with the multidimensional scores but differed in the two analyses suggesting no dominant effect by particular alleles at any locus. Both the 1996 and 1986 outbreak sets showed no significant population differentiation and large N_em values that were significantly greater than that for the 1986 non-outbreak set (Table 3).

The close clustering in genetic space between both the 1996 and 1986 sets of outbreak populations suggest that both were derived from sources with similar gene frequencies. The higher average number of migrants between populations in the 1996 outbreak set may reflect the greater geographic proximity of the 1996 outbreak populations (maximum separation approximately 130 km) compared with the 1986 outbreak set (maximum separation approximately 800 km). Benzie and Stoddart (1992) noted that ENOL*85, although missing from two of the eight outbreak populations, was only found in outbreak populations. It was so

rare (frequencies of 0.01-0.02) that it might not be expected to be found in the smaller samples from the five non-outbreak populations. However, it is pertinent to note that all the 1996 outbreak populations possessed *ENOL*85* at frequencies of 0.02-0.05, consistent with the 1996 outbreak populations having been derived from the same source as the 1986 outbreak populations.

In analyses where all outbreak populations were kept separate, F_{ST} values were not significant, suggesting the sets of outbreak populations were not genetically differentiated (Table 4). However, when the 1996 and 1986 sets of outbreak populations were each pooled to provide a single pairwise comparison, the mean F_{ST} value was highly significant. Statistically significant but small differences in gene frequencies were thought to result from genetic drift (see section on within population structure below), but are unlikely to provide a means of distinguishing sets of outbreak populations because of the large sample sizes required to detect the small shifts in gene frequency.

4.3 Within population structure

Stochastic variation in reproductive success, either through differential fecundity, differential fertilisation success, differences in larval survival, settlement success or post-settlement survival, along with chance effects on which genotypes mate, can change gene frequencies (i.e. genetic drift) (Spiess 1989). It is possible to use these changes where they are strong enough, to trace the number or geographical derivation of recruits (Johnson and Black 1984; Kordos and Burton 1993). Where the number of adults contributing to the next generation is as high as in *Acanthaster planci* the shifts in gene frequency as a result of genetic drift are likely to be small and detectable only at very large sample sizes.

Table 1. Population differentiation, measured by F_{ST}, for each age class. No calculation of F_{ST} was made for age classes 1, 8, and 9 because of the small number of populations and small mean sample size of each population. The spawning season from which the year class was derived is given in parentheses below the age class.

	Age class								
	1 (94/95)	2 (93/94)	3 (92/93)	4 (91/92)	5 (90/91)	6 (89/90)	7 (88/89)	8 (87/88)	9 (86/87)
Number of populations	3	6	6	6	6	5	4	3	1
Mean sample size per population	1.7 (0.4)	42.2 (8.2)	47.8 (8.1)	44.2 (15.9)	16.3 (5.7)	8.0 (3.6)	4.0 (1.2)	2.3 (1.4)	4 (0)
F_{ST}	-	0.000^{NS}	0.003^{NS}	-0.003^{NS}	-0.006^{NS}	0.012^{NS}	0.113^{NS}	-	-

NS = not significant.

Table 2. Population differentiation, measured by F_{ST}, between each age class. No calculation of F_{ST} was made for age classes where the sample size for either of the populations was less than 4.

	Age classes compared				
Population	2-3	3-4	4-5	5-6	6-7
Lizard Island	-0.002^{NS}	0.002^{NS}	0.006^{NS}	-0.022^{NS}	-0.023^{NS}
Heldston	-0.002^{NS}	-0.034^{NS}	-0.057^{NS}	-0.042^{NS}	-
Two Isles	0.000^{NS}	0.001^{NS}	0.054^{NS}	0.027^{NS}	-
Endeavour	0.009^{NS}	-0.002^{NS}	-0.006^{NS}	-	-
Pickersgill	-0.001^{NS}	0.001^{NS}	-0.003^{NS}	-0.069^{NS}	-0.046^{NS}
Evening	-0.005^{NS}	-0.003^{NS}	-0.003^{NS}	-0.005^{NS}	-0.047^{NS}

NS = not significant.

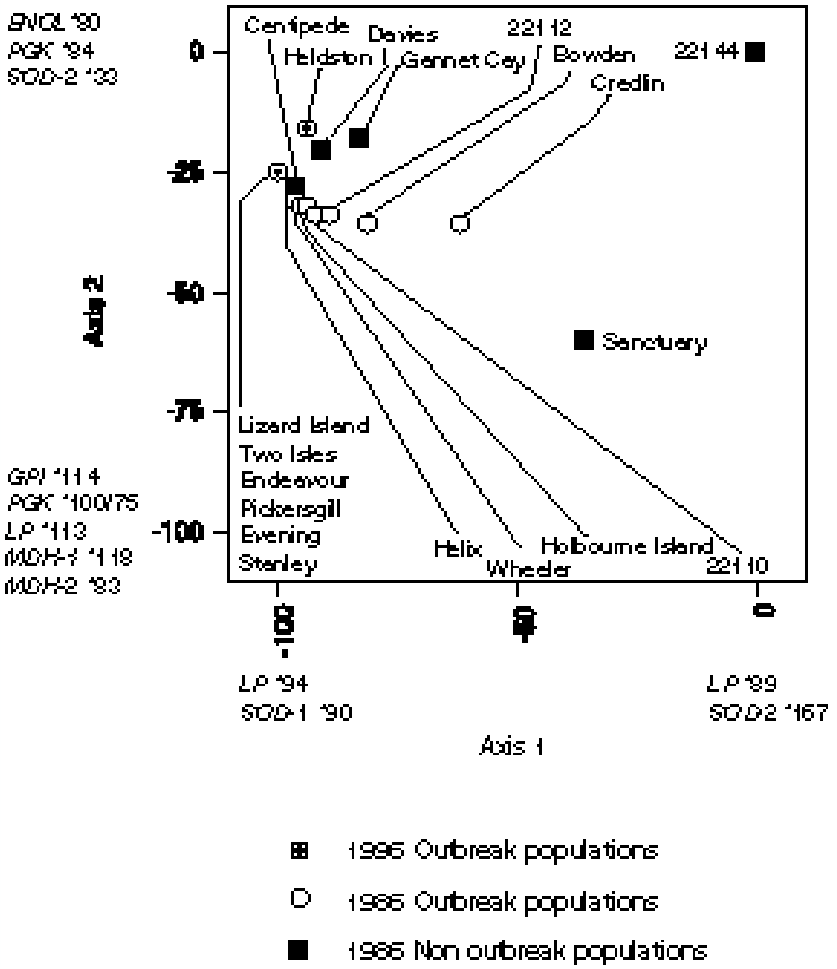

Figure 2. Multidimensional scaling plot of outbreak populations and non-outbreak populations in genetic space, including data from *MDH-1**.

Table 3. F-statistics describing population differentiation in sets of outbreak and non-outbreak populations from the Great Barrier Reef, together with estimates of average gene flow among populations within each set. Data for the 1986 populations are from Benzie and Stoddart (1992).

	Outbreak		Non-outbreak
	1996	1986	1986
Number of populations sampled	6	8	5
Unbiased estimate of Fst (?95% confidence limits)	0.001^{NS} (?0.0001)	0.007^{NS} (?0.0002)	0.020^{**} (?0.002)
Average number of migrants per generation (N_em) and (95% confidence limits)	249.8 (227.0-277.5)	35.5 (27.5-49.8)	12.3 (11.1-13.6)

** $P < 0.01$, NS = not significant.

Table 4. Standardised variance within (F_{IS}) and between (F_{ST}) populations, and the total variance in allele frequencies (F_{TOT}) for all (1996 and 1986) outbreak populations. All populations were kept separate in the first analysis, and all 1996 outbreak populations were pooled and compared to the pooled 1986 outbreak population set in the second analysis. Data for the 1986 populations are from Benzie and Stoddart (1992). A number of cells had low expected values, but analyses pooling rare alleles did not alter the nature of the results.

	Populations separate (14 pops)			Populations pooled (2 pops)		
Locus	F_{IS}	F_{TOT}	F_{ST}	F_{IS}	F_{TOT}	F_{ST}
*GPI**	0.105^{***}	0.115	0.011^{***}	0.106^{***}	0.119	0.015^{***}
*ENOL**	0.000^{NS}	0.003	0.003^{NS}	0.003^{NS}	0.004	0.001^{**}
*PGK**	0.122^{***}	0.122	-0.001^{NS}	0.121^{***}	0.121	0.000^{NS}
*LT-3**	0.041^{NS}	0.041	0.000^{NS}	0.041^{NS}	0.043	0.002^{**}
*LP**	0.115^{***}	0.121	0.006^{NS}	0.116^{***}	0.126	0.011^{***}
*SOD-1**	0.021^{NS}	0.028	0.007^{*}	0.027^{NS}	0.028	0.001^{*}
*SOD-2**	-0.008^{NS}	-0.001	0.007^{*}	-0.001^{NS}	-0.002	0.000^{NS}
*MDH-1**	0.209^{***}	0.220	0.014^{***}	0.214^{***}	0.219	0.007^{***}
*MDH-2**	-0.032^{NS}	-0.031	0.002^{NS}	-0.030^{NS}	-0.029	0.001^{*}
Average	0.064^{**}	0.069	0.005^{NS}	0.066^{**}	0.070	0.004^{***}

* P < 0.05, ** P < 0.01, *** P < 0.001, NS = not significant.

Table 5. ?^{2} values testing for conformance of gene frequencies to those expected under conditions of Hardy-Weinberg Equilibrium, and pooling rare alleles (degrees of freedom for all tests is therefore 1). The sign indicates whether there was a deficit (-) or an excess (+) of heterozygotes. M indicates the locus was monomorphic in that population.

Locus	Lizard Island	Heldston	Two Isles	Endeavour	Pickersgill	Evening
*GPI**	2.22^{NS}(-)	7.81^{NS}(-)	1.13^{NS}(-)	1.13^{NS}(-)	1.06^{NS}(+)	$0\ .01^{NS}$(-)
*ENOL**	0.25^{NS}(+)	4.97^{NS}(-)	0.06^{NS}(+)	0.06^{NS}(+)	0.39^{NS}(+)	4.45^{NS}(-)
*PGK**	3.46^{NS}(-)	**15.24^{*} (-)**	0.10^{NS}(+)	0.64^{NS}(-)	0.51^{NS}(-)	1.37^{NS}(-)
*LT-3**	0.96^{NS}(-)	5.22^{NS}(-)	0.19^{NS}(-)	0.01^{NS}(+)	0.01^{NS}(+)	0.64^{NS}(-)
*LP**	3.07^{NS}(-)	0.01^{NS}(-)	0.60^{NS}(-)	8.42^{NS}(-)	4.09^{NS}(-)	**18.28^{*} (-)**
*SOD-1**	0.03^{NS}(+)	0.04^{NS}(-)	0.72^{NS}(+)	0.07^{NS}(-)	0.15^{NS}(-)	0.13^{NS}(-)
*SOD-2**	M	M	0.01^{NS}(+)	M	M	0.00^{NS}(+)
*MDH-1**	0.98^{NS}(-)	**10.11^{*} (-)**	**45.10^{*} (-)**	7.00^{NS}(-)	0.09^{NS}(+)	**13.18^{*} (-)**

*MDH-2**	2.25^{NS}(+)	0.15^{NS}(+)	0.04^{NS}(-)	0.51^{NS}(-)	1.48^{NS}(-)	3.02^{NS}(+)

* P < 0.05, NS = not significant.

In every population the observed heterozygosity did not deviate significantly from that expected under conditions of Hardy-Weinberg equilibrium (i.e. conditions of random mating). Statistically significant values for FIS at PGK*, LP* and MDH-1* suggested some intrapopulation structuring with heterozygote deficits at these loci (Table 5). However, the mean FIS was not significant, suggesting no strong and consistent internal population structuring. Only five marginally significant deviations from Hardy-Weinberg gene frequencies were observed in ?2 tests, pooling rare alleles (Table 5). All of the deviations were heterozygote deficits, three of which (MDH-1* at Heldston, Two Isles and Evening reef populations) represented only minor deviations and were unlikely to be biologically significant. Two others (LP* in the Evening reef population and PGK* in the Heldston reef population) each demonstrated 15-25 more homozygotes more than expected suggesting some degree of heterogeneity of gene frequencies among the recruits to these populations. However, only *PGK** showed significant deviations when exact tests (Elston and Forthofer 1977) were applied (results not shown here).

F_{IS} values were also significant, and similar, in both analyses comparing the 1986 and 1996 outbreak populations, reflecting some intrapopulation structure at *GPI**, *PGK**, *LP**, and *MDH-1** (Table 4).

The marginally significant deficits of heterozygotes are likely to reflect genetic drift resulting from variance in reproductive success and chance effects determining which genotypes mate, giving rise to slight differences in gene frequencies between different individual recruitment events. However, none of these effects was so strong as to lead to clearly differentiated populations on the temporal and spatial scales investigated and which might be used to determine in more detail the geographical origin of recruits within the region.

4.4 Evidence of patterns of recruitment

The major proportion (70-80%) of individuals were aged between 2 and 4 years old on each reef (Fig. 3). It is tempting to infer a later increase in recruitment on the northern reefs given that two of these (Heldston Reef and Two Isles) had a higher proportion of 2- and 3-year olds relative to the southern reefs which had high proportions of 2- to 4-year olds. However, the most northerly reef, Lizard Island, had an age structure comparable to the southern reefs (see also Stump 1996), and a more exhaustive study of the age structure of 25 reefs between latitudes 14-16?S showed no relationship between latitude and inferred start of high

recruitment (Engelhardt and Lassig (in press)). These data suggest increased recruitment occurred throughout the region 14-16?S, although populations on some reefs increased before others. The genetic data suggest that all populations derived their recruits from a common pool of gametes in that region, probably derived from several source reefs.

In summary, allozyme analysis has provided evidence that the populations of *Acanthaster planci* between 14-16?S showing increases in population size in the early 1990s are likely to have derived their recruits from the same gene pool since 1989. This is likely to have been the same source as that giving rise to the 1986 outbreak populations. Small stochastic shifts in gene frequencies as a result of genetic drift were identified from evidence of some intra-population structuring, and from differences between the pooled 1996 and pooled 1986 outbreak populations, but these were insufficient to resolve dispersal events among the populations in any more detail.

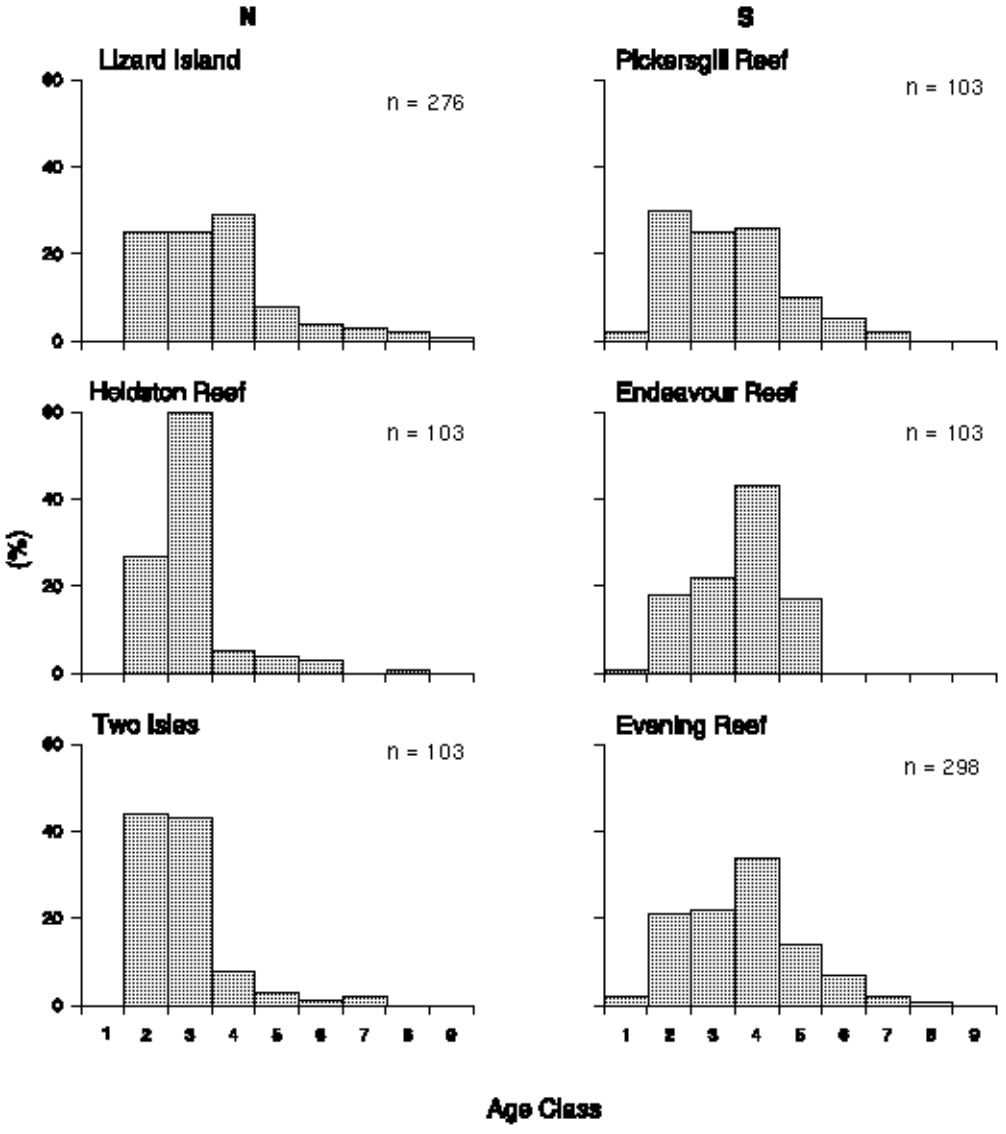

Figure 3. Proportion of individuals in different age classes for each of the six populations sampled.

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7. APPENDIX

Allele frequencies of the six populations, pooling all age classes (Table A1), and for each age class in each population (Tables A2-A7). Data on genetic diversity of the six populations (Table A8), and for each age class in each population (Tables A9-A14).

Table A1. Allele frequencies in six 1996 outbreak populations pooling all age classes.

	Population					
Locus, allele	Lizard Island	Heldston	Two Isles	Endeavour	Pickersgill	Evening
*GPI**						
121	-	-	-	-	-	0.002
114	0.004	0.005	0.024	0.005	0.015	0.034
107	0.040	0.044	0.039	0.049	0.015	0.023
100	0.864	0.869	0.869	0.869	0.908	0.867
93	0.067	0.078	0.049	0.058	0.044	0.062
86	0.025	0.005	0.015	0.019	0.019	0.010
71	-	-	-	-	-	0.002
57	-	-	0.005	-	-	-
*ENOL**						
113	-	0.005	0.005	0.005	-	-
110	0.004	-	-	-	-	-
105	-	0.005	-	-	-	-
100	0.971	0.961	0.976	0.976	0.942	0.977
90	-	0.005	-	-	-	0.002
85	0.022	0.015	0.019	0.019	0.053	0.018
80	0.004	0.010	-	-	-	0.003
70	-	-	-	-	0.005	-
*PGK**						
109	-	0.010	-	-	0.005	0.003
100	0.556	0.646	0.568	0.549	0.568	0.540
91	0.377	0.301	0.364	0.374	0.379	0.383
84	0.067	0.044	0.068	0.078	0.049	0.072
75	-	-	-	-	-	0.002
*LT-3**						
110	0.203	0.165	0.204	0.223	0.223	0.220
100	0.797	0.835	0.796	0.777	0.777	0.779
90	-	-	-	-	-	0.002
*LP**						
124	0.007	0.010	0.019	0.010	0.010	0.013
120	0.020	0.044	0.039	0.019	0.024	0.015
113	0.185	0.131	0.175	0.184	0.155	0.198
111	0.002	0.005	0.005	0.024	0.005	0.022
107	0.114	0.073	0.092	0.107	0.068	0.057
103	0.016	0.019	0.010	0.024	0.024	0.027
100	0.605	0.670	0.617	0.573	0.617	0.626
94	0.051	0.049	0.039	0.058	0.078	0.035
89	-	-	0.005	-	0.019	0.007
*SOD-1**						
100	0.935	0.869	0.859	0.913	0.917	0.879
90	0.065	0.131	0.141	0.087	0.083	0.121
*SOD-2**						
100	1.000	1.000	0.990	1.000	1.000	0.998
33	-	-	0.010	-	-	0.002
*MDH-1**						
118	0.002	0.010	-	-	-	-
114	0.033	0.015	0.015	0.034	0.029	0.013
100	0.962	0.971	0.985	0.966	0.971	0.985
93	0.002	-	-	-	-	-
82	0.002	0.005	-	-	-	0.002
*MDH-2**						

120	0.004	-	-	0.005	-	-
113	0.004	-	0.010	0.015	-	0.007
100	0.888	0.883	0.869	0.888	0.874	0.883
88	-	-	-	-	0.005	0.002
75	0.105	0.117	0.121	0.092	0.121	0.109
Sample size	276	103	103	103	103	298

Table A2. Allele frequencies for each age class in the Lizard Island population.

Locus, alleles	Age class							
	2	3	4	5	6	7	8	9
*GPI**								
114	-	0.007	0.006	-	-	-	-	-
107	0.036	0.015	0.062	0.024	0.100	0.071	0.100	-
100	0.855	0.853	0.852	0.905	0.850	0.857	0.900	1.000
93	0.072	0.066	0.074	0.071	0.050	0.071	-	-
86	0.036	0.059	0.006	-	-	-	-	-
*ENOL**								
110	0.007	0.007	-	-	-	-	-	-
100	0.978	0.963	0.988	0.976	0.950	1.000	0.900	0.625
85	0.014	0.022	0.012	-	0.050	-	0.100	0.375
80	-	0.007	-	0.024	-	-	-	-
*PGK**								
100	0.514	0.551	0.574	0.619	0.550	0.714	0.700	0.625
91	0.406	0.375	0.358	0.357	0.450	0.286	0.200	0.375
84	0.080	0.074	0.068	0.024	-	-	0.100	-
*LT-3**								
110	0.174	0.132	0.247	0.286	0.150	0.214	0.400	0.375
100	0.826	0.868	0.753	0.714	0.850	0.786	0.600	0.625
*LP**								
124	0.014	-	0.006	-	-	0.071	-	-
120	0.036	0.022	0.006	0.024	-	-	0.100	-
113	0.152	0.191	0.216	0.238	0.050	0.143	0.200	0.250
111	-	-	-	0.024	-	-	-	-
107	0.145	0.110	0.068	0.190	0.100	-	0.200	0.375
103	0.014	0.015	0.012	0.024	0.100	-	-	-
100	0.580	0.581	0.660	0.476	0.600	0.786	0.500	0.375
94	0.058	0.081	0.031	0.024	0.150	-	-	-
*SOD-1**								
100	0.920	0.956	0.944	0.929	1.000	0.929	0.900	0.750
90	0.080	0.044	0.056	0.071	-	0.071	0.100	0.250
*SOD-2**								
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*MDH-1**								
118	-	0.007	-	-	-	-	-	-
114	0.029	0.037	0.031	0.048	-	-	-	-
100	0.971	0.949	0.969	0.952	0.950	1.000	1.000	1.000
93	-	-	-	-	0.050	-	-	-
82	-	0.007	-	-	-	-	-	-
*MDH-2**								
120	-	0.007	0.006	-	-	-	-	-
113	-	0.007	-	-	-	-	-	-
100	0.884	0.912	0.914	0.762	0.800	0.857	0.800	1.000
75	0.116	0.074	0.080	0.238	0.200	0.143	0.200	-
Sample size	69	68	81	21	10	7	5	4

Table A3. Allele frequencies for each age class in the Heldston Reef population.

Locus, alleles	Age class					
	2	3	4	5	6	8
*GPI**						
114	-	0.008	-	-	-	-
107	0.036	0.016	0.200	0.125	0.167	0.500
100	0.893	0.911	0.800	0.875	0.167	-
93	0.071	0.056	-	-	0.667	0.500
86	-	0.008	-	-	-	-
*ENOL**						
113	-	-	-	0.125	-	-
105	-	0.008	-	-	-	-
100	0.982	0.952	1.000	0.875	1.000	1.000
90	-	0.008	-	-	-	-
85	0.018	0.016	-	-	-	-
80	-	0.016	-	-	-	-
*PGK**						
109	0.018	-	-	0.125	-	-
100	0.625	0.661	0.600	0.750	0.667	-
91	0.286	0.298	0.400	0.125	0.333	1.000
84	0.071	0.040	-	-	-	-
*LT-3**						
110	0.089	0.194	0.200	0.250	0.167	-
100	0.911	0.806	0.800	0.750	0.833	1.000
*LP**						
124	-	0.016	-	-	-	-
120	-	0.040	0.100	0.125	0.167	0.500
113	0.161	0.129	-	0.125	0.167	-
111	-	0.008	-	-	-	-
107	0.089	0.056	0.200	0.125	-	-
103	0.018	0.024	-	-	-	-
100	0.714	0.653	0.700	0.625	0.667	0.500
94	0.018	0.073	-	-	-	-
*SOD-1**						
100	0.804	0.895	0.700	1.000	1.000	1.000
90	0.196	0.105	0.300	-	-	-
*SOD-2**						
100	1.000	1.000	1.000	1.000	1.000	1.000
*MDH-1**						
118	0.036	-	-	-	-	-
114	0.018	0.016	-	-	-	-
100	0.946	0.976	1.000	1.000	1.000	1.000
82	-	0.008	-	-	-	-
*MDH-2**						
100	0.911	0.871	0.800	1.000	0.833	1.000
75	0.089	0.129	0.200	-	0.167	-
Sample size	28	62	5	4	3	2

Table A4. Allele frequencies for each age class in the Two Isles population.

Locus, alleles	Age class					
	2	3	4	5	6	7
*GPI**						
114	0.022	0.034	-	-	-	-
107	0.044	0.045	-	-	-	-
100	0.833	0.909	0.813	1.000	1.000	0.750
93	0.067	0.011	0.125	-	-	0.250
86	0.033	-	-	-	-	-
57	-	-	0.063	-	-	-
*ENOL**						
113	-	-	-	0.167	-	-
100	0.978	0.977	1.000	0.833	1.000	1.000
85	0.022	0.023	-	-	-	-
*PGK**						
100	0.567	0.568	0.813	0.167	1.000	-
91	0.344	0.386	0.125	0.667	-	1.000
84	0.089	0.045	0.063	0.167	-	-
*LT-3**						
110	0.211	0.216	0.125	-	0.500	0.250
100	0.789	0.784	0.875	1.000	0.500	0.750
*LP**						
124	0.022	0.023	-	-	-	-
120	0.022	0.068	-	-	-	-
113	0.167	0.193	0.188	0.167	-	-
111	-	0.011	-	-	-	-
107	0.089	0.091	0.063	0.167	-	0.250
103	0.011	0.011	-	-	-	-
100	0.644	0.545	0.750	0.667	1.000	0.750
94	0.044	0.045	-	-	-	-
89	-	0.011	-	-	-	-
*SOD-1**						
100	0.867	0.841	0.938	0.833	0.500	1.000
90	0.133	0.159	0.063	0.167	0.500	-
*SOD-2**						
100	0.989	1.000	1.000	1.000	1.000	0.750
33	0.011	-	-	-	-	0.250
*MDH-1**						
114	0.033	-	-	-	-	-
100	0.967	1.000	1.000	1.000	1.000	1.000
*MDH-2**						
113	-	0.023	-	-	-	-
100	0.911	0.841	0.875	0.667	0.500	1.000
75	0.089	0.136	0.125	0.333	0.500	-
Sample size	45	44	8	3	2	2

Table A5. Allele frequencies for each age class in the Endeavour population.

Locus, alleles	Age class				
	1	2	3	4	5
*GPI**					
114	-	-	0.022	-	-
107	-	0.056	0.043	0.057	0.029
100	1.000	0.889	0.826	0.909	0.794
93	-	0.056	0.109	0.011	0.118
86	-	-	-	0.023	0.059
*ENOL**					
113	-	-	-	0.011	-
100	1.000	0.972	1.000	0.966	0.971
85	-	0.028	-	0.023	0.029
*PGK**					
100	1.000	0.500	0.630	0.523	0.529
91	-	0.389	0.304	0.398	0.412
84	-	0.111	0.065	0.080	0.059
*LT-3**					
110	0.500	0.111	0.304	0.216	0.235
100	0.500	0.889	0.696	0.784	0.765
*LP**					
124	-	-	0.022	0.011	-
120	-	0.028	0.022	0.011	0.029
113	0.500	0.111	0.174	0.216	0.176
111	-	0.028	0.043	0.023	-
107	0.500	0.111	0.087	0.091	0.147
103	-	-	0.043	0.034	-
100	-	0.667	0.565	0.523	0.647
94	-	0.056	0.043	0.091	-
*SOD-1**					
100	1.000	0.889	0.935	0.898	0.941
90	-	0.111	0.065	0.102	0.059
*SOD-2**					
100	1.000	1.000	1.000	1.000	1.000
*MDH-1**					
114	-	0.056	0.043	0.034	-
100	1.000	0.944	0.957	0.966	1.000
*MDH-2**					
120	-	-	-	0.011	-
113	-	-	-	0.011	0.059
100	1.000	0.917	0.870	0.875	0.912
75	-	0.083	0.130	0.102	0.029
Sample size	2	18	23	44	17

Table A6. Allele frequencies for each age class in the Pickersgill population.

Locus, alleles	Age Class 1	2	3	4	5	6	7
*GPI**							
114	-	0.016	-	0.037	-	-	-
107	-	0.016	0.019	-	0.050	-	-
100	0.750	0.903	0.923	0.889	0.900	1.000	1.000
93	0.250	0.032	0.038	0.056	0.050	-	-
86	-	0.032	0.019	0.019	-	-	-
*ENOL**							
100	1.000	0.903	0.962	1.000	0.950	0.900	0.500
85	-	0.081	0.038	-	0.050	0.100	0.500
70	-	0.016	-	-	-	-	-
*PGK**							
109	-	-	0.019	-	-	-	-
100	0.250	0.500	0.654	0.537	0.600	0.800	0.500
91	0.750	0.435	0.327	0.389	0.400	0.100	0.250
84	-	0.065	-	0.074	-	0.100	0.250
*LT-3**							
110	-	0.226	0.212	0.278	0.200	0.100	0.250
100	1.000	0.774	0.788	0.722	0.800	0.900	0.750
*LP**							
124	-	0.032	-	-	-	-	-
120	-	0.016	0.019	0.056	-	-	-
113	0.250	0.113	0.192	0.204	0.100	0.100	-
111	-	0.016	-	-	-	-	-
107	-	0.081	0.038	0.074	0.100	0.100	-
103	0.250	0.016	0.019	0.019	-	-	0.250
100	0.500	0.677	0.615	0.574	0.500	0.700	0.750
94	-	0.016	0.096	0.056	0.300	0.100	-
89	-	0.032	0.019	0.019	-	-	-
*SOD-1**							
100	1.000	0.871	0.981	0.926	0.850	0.900	1.000
90	-	0.129	0.019	0.074	0.150	0.100	-
*SOD-2**							
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*MDH-1**							
114	-	0.016	0.019	0.056	0.050	-	-
100	1.000	0.984	0.981	0.944	0.950	1.000	1.000
*MDH-2**							
100	1.000	0.839	0.865	0.907	0.850	0.900	1.000
88	-	-	-	0.019	-	-	-
75	-	0.161	0.135	0.074	0.150	0.100	-
Sample size	2	31	26	27	10	5	2

Table A7. Allele frequencies for each age class in the Evening population.

Locus, allele	Age Class 1	2	3	4	5	6	7	8
*GPI**								
121	-	-	-	-	-	0.024	-	-
114	-	0.016	0.039	0.045	0.012	0.048	-	0.500
107	-	0.024	0.031	0.025	0.023	-	-	-
100	1.000	0.855	0.852	0.870	0.872	0.905	1.000	0.500
93	-	0.097	0.047	0.055	0.081	0.024	-	-
86	-	0.008	0.031	-	0.012	-	-	-
71	-	-	-	0.005	-	-	-	-
*ENOL**								
100	0.750	0.984	0.984	0.970	0.988	0.976	1.000	0.500
90	-	-	-	-	-	-	-	0.500
85	0.250	0.016	0.008	0.025	0.012	0.024	-	-
80	-	-	0.008	0.005	-	-	-	-
*PGK**								
109	-	-	-	0.005	0.012	-	-	-
100	0.250	0.565	0.500	0.560	0.512	0.595	0.400	1.000
91	0.750	0.395	0.414	0.370	0.349	0.333	0.500	-
84	-	0.040	0.086	0.065	0.116	0.071	0.100	-
75	-	-	-	-	0.012	-	-	-
*LT-3**								
110	0.500	0.226	0.219	0.210	0.244	0.190	0.200	-
100	0.500	0.774	0.773	0.790	0.756	0.810	0.800	1.000
90	-	-	0.008	-	-	-	-	-
*LP**								
124	-	0.016	0.016	0.015	0.012	-	-	-
120	-	0.016	-	0.020	0.012	0.048	-	-
113	-	0.202	0.180	0.225	0.198	0.143	0.200	-
111	-	0.056	0.016	-	0.035	0.024	-	-
107	-	0.040	0.070	0.050	0.081	0.048	0.100	-
103	-	0.032	0.016	0.030	0.012	0.048	0.100	-
100	1.000	0.597	0.648	0.625	0.605	0.667	0.500	1.000
94	-	0.032	0.047	0.035	0.035	0.024	-	-
89	-	0.008	0.008	-	0.012	-	0.100	-
*SOD-1**								
100	1.000	0.847	0.867	0.880	0.907	0.929	0.900	1.000
90	-	0.153	0.133	0.120	0.093	0.071	0.100	-
*SOD-2**								
100	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000
33	-	-	-	-	0.012	-	-	-
*MDH-1**								
114	-	0.008	0.031	0.010	-	0.024	-	-
100	1.000	0.984	0.969	0.990	1.000	0.976	1.000	1.000
82	-	0.008	-	-	-	-	-	-
*MDH-2**								
113	-	0.008	0.016	-	0.012	-	-	-
100	1.000	0.871	0.867	0.890	0.872	0.929	0.900	1.000
88	-	-	-	0.005	-	-	-	-
75	-	0.121	0.117	0.105	0.116	0.071	0.100	-
Sample size	2	62	64	100	43	21	5	2

Table A8. Genetic diversity measures for all six populations (pooling all age classes). The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
Lizard	276.0 (0.0)	3.8 (0.7)	88.9	0.226 (0.063)	0.239 (0.070)
Heldston	103.0 (0.0)	3.8 (0.8)	88.9	0.201 (0.053)	0.234 (0.061)
Two Isles	103.0 (0.0)	3.6 (0.8)	100.0	0.239 (0.066)	0.251 (0.069)
Endeavour	103.0 (0.0)	3.3 (0.7)	88.9	0.233 (0.068)	0.250 (0.074)
Pickersgill	103.0 (0.0)	3.4 (0.8)	88.9	0.228 (0.061)	0.243 (0.069)
Evening	298.0 (0.0)	4.3 (0.8)	100.0	0.228 (0.062)	0.246 (0.070)

Table A9. Genetic diversity measures for each age class represented in the Lizard Island population. The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
Lizard 2 year	69.0 (0.0)	2.9 (0.6)	88.9	0.229 (0.064)	0.244 (0.074)
Lizard 3 year	68.0 (0.0)	3.4 (0.5)	88.9	0.229 (0.070)	0.232 (0.072)
Lizard 4 year	81.0 (0.0)	3.0 (0.6)	88.9	0.203 (0.059)	0.227 (0.069)
Lizard 5 year	21.0 (0.0)	2.7 (0.6)	88.9	0.254 (0.077)	0.271 (0.079)
Lizard 6 year	10.0 (0.0)	2.2 (0.4)	77.8	0.222 (0.060)	0.248 (0.074)
Lizard 7 year	7.0 (0.0)	1.9 (0.3)	66.7	0.238 (0.071)	0.208 (0.059)
Lizard 8 year	5.0 (0.0)	2.1 (0.3)	77.8	0.333 (0.105)	0.304 (0.083)
Lizard 9 year	4.0 (0.0)	1.7 (0.2)	55.6	0.333 (0.118)	0.310 (0.102)

Table A10. Genetic diversity measures for each age class represented in the Heldston Reef population. The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
2 year	28.0 (0.0)	2.7 (0.4)	88.9	0.198 (0.057)	0.221 (0.061)
3 year	62.0 (0.0)	3.4 (0.7)	88.9	0.197 (0.050)	0.230 (0.062)
4 year	5.0 (0.0)	1.8 (0.2)	66.7	0.200 (0.082)	0.286 (0.075)
5 year	4.0 (0.0)	1.9 (0.4)	55.6	0.194 (0.091)	0.226 (0.081)
6 year	3.0 (0.0)	1.8 (0.3)	55.6	0.296 (0.103)	0.267 (0.090)
8 year	2.0 (0.0)	1.2 (0.1)	22.2	0.222 (0.147)	0.148 (0.098)

Table A11. Genetic diversity measures for each age class represented in the Two Isles Reef population. The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
2 year	45.0 (0.0)	3.0 (0.6)	100.0	0.235 (0.065)	0.253 (0.068)
3 year	44.0 (0.0)	3.0 (0.8)	77.8	0.247 (0.077)	0.255 (0.077)
4 year	8.0 (0.0)	2.0 (0.3)	66.7	0.194 (0.059)	0.189 (0.055)
5 year	3.0 (0.0)	1.8 (0.3)	55.6	0.296 (0.103)	0.267 (0.090)
6 year	2.0 (0.0)	1.3 (0.2)	33.3	0.333 (0.167)	0.222 (0.111)
7 year	2.0 (0.0)	1.4 (0.2)	44.4	0.222 (0.088)	0.222 (0.088)

Table A12. Genetic diversity measures for each age class represented in the Endeavour Reef population. The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
1 year	2.0 (0.0)	1.2 (0.1)	22.2	0.222 (0.147)	0.148 (0.098)
2 year	18.0 (0.0)	2.6 (0.5)	88.9	0.222 (0.067)	0.231 (0.069)
3 year	23.0 (0.0)	2.8 (0.7)	77.8	0.275 (0.081)	0.261 (0.078)
4 year	44.0 (0.0)	3.2 (0.7)	88.9	0.227 (0.066)	0.255 (0.077)
5 year	17.0 (0.0)	2.4 (0.4)	77.8	0.203 (0.072)	0.242 (0.074)

Table A13. Genetic diversity measures for each age class represented in thePickersgill Reef population. The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
1 year	2.0 (0.0)	1.4 (0.2)	33.3	0.167 (0.083)	0.204 (0.107)
2 year	31.0 (0.0)	3.2 (0.8)	88.9	0.244 (0.068)	0.261 (0.065)
3 year	26.0 (0.0)	2.8 (0.6)	88.9	0.201 (0.060)	0.215 (0.070)
4 year	27.0 (0.0)	2.8 (0.6)	77.8	0.235 (0.067)	0.248 (0.078)
5 year	10.0 (0.0)	2.2 (0.3)	88.9	0.256 (0.065)	0.272 (0.071)
6 year	5.0 (0.0)	2.0 (0.3)	66.7	0.178 (0.052)	0.190 (0.060)
7 year	2.0 (0.0)	1.6 (0.2)	44.4	0.278 (0.121)	0.278 (0.115)

Table A14. Genetic diversity measures for each age class represented in the Evening Reef population. The expected diversity is that expected under conditions of Hardy-Weinberg Equilibrium. A locus was considered polymorphic if one variant was observed. Standard deviations on the mean are given (in parentheses) beneath the mean where appropriate.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent loci polymorphic	Mean Heterozygosity	
				Direct count	Expected
1 year	2.0 (0.0)	1.3 (0.2)	33.3	0.222 (0.121)	0.185 (0.094)
2 year	62.0 (0.0)	3.3 (0.8)	88.9	0.231 (0.057)	0.255 (0.072)
3 year	64.0 (0.0)	3.3 (0.7)	88.9	0.245 (0.065)	0.256 (0.070)
4 year	100.0 (0.0)	3.2 (0.6)	88.9	0.222 (0.067)	0.241 (0.069)
5 year	43.0 (0.0)	3.4 (0.8)	88.9	0.222 (0.067)	0.251 (0.078)
6 year	21.0 (0.0)	2.8 (0.6)	88.9	0.212 (0.066)	0.216 (0.069)
7 year	5.0 (0.0)	2.0 (0.4)	55.6	0.222 (0.091)	0.240 (0.097)
8 year	2.0 (0.0)	1.2 (0.1)	22.2	0.222 (0.147)	0.148 (0.098)