85/8

FISHING INDUSTRY RESEARCH DEVELOPMENT COUNCIL
FINAL REPORT (F.I.R.T.A. 85/8)

1. TITLE OF PROPOSAL
Breeding structure and stock identification in blacklip and greenlip abalone (*Haliotis* spp.).

2. NAME OF APPLICANT
La Trobe University.

3. DIVISION, SECTION OR DEPARTMENT
Department of Genetics and Human Variation.

4. PROPOSAL
To investigate genetic variation and breeding structure in populations of blacklip and greenlip abalone (*Haliotis* spp.). Morphological and electrophoretic information will be used to identify and delineate discrete breeding populations and so allow more informed assessment of stock/recruitment relationships and hence yield in the major south-eastern abalone fisheries.

5. NAME OF PERSON RESPONSIBLE FOR PROGRAM
Dr. N.D. Murray.

6. QUALIFICATIONS OF STAFF TO BE EMPLOYED ON PROGRAM
(a) Dr N.D. Murray, BSc, PhD, (Sydney), Senior Lecturer in Genetics (Principle Supervisor).

(b) Dr C.M. MacDonald, BSc, MSc, PhD. (ANU), Fisheries Assessment Officer, Conservation, Forests and Lands, Victoria; Division of of Fisheries and Wildlife (Associate Supervisor).

(c) Mr L. Brown, BSc. (Hons, Division 2A).
The project will form the basis of a doctoral dissertation by Mr Brown.

7. OBJECTIVES
(a) To determine the dispersal capabilities and breeding structure of blacklip and greenlip abalone stocks exploited by fisheries in South Australian, Tasmanian, Victorian and NSW waters.

(b) To determine whether, and to what extent, interbreeding between blacklip and greenlip abalone occurs.

(c) To obtain information on the spatial and temporal distribution of genetic variation in blacklip and greenlip abalone which will be of vital importance in the development of future artificial culture and reef enhancement programs.

(d) To provide the above information to fisheries managers and to liase with them to facilitate better assessments of stock/recruitment relationships, and hence sustainable yield, in the major south-eastern abalone fisheries.

A. To determine the dispersal capabilities and breeding structure of blacklip and greenlip abalone stocks exploited by fisheries in South Australian, Tasmanian, Victorian and NSW waters.

In all 1,431 adult *H.rubra* from seventeen locations (ave. sample size 84.18) have been collected, this includes a sample of *H. conicopora* from Cape Arid, Western Australia (WCA) which will be regarded as synonymous with *H. rubra* in this study (Brown and Murray 1990b). A total of 614 *H. laevigata* from nine locations* (ave. sample size 68.22) have been collected. Collection sites are shown in Figure 1. Genetic analyses were based on twelve polymorphic and three monomorphic loci in *H. rubra*, thirteen polymorphic and two monomorphic loci in *H. laevigata*.

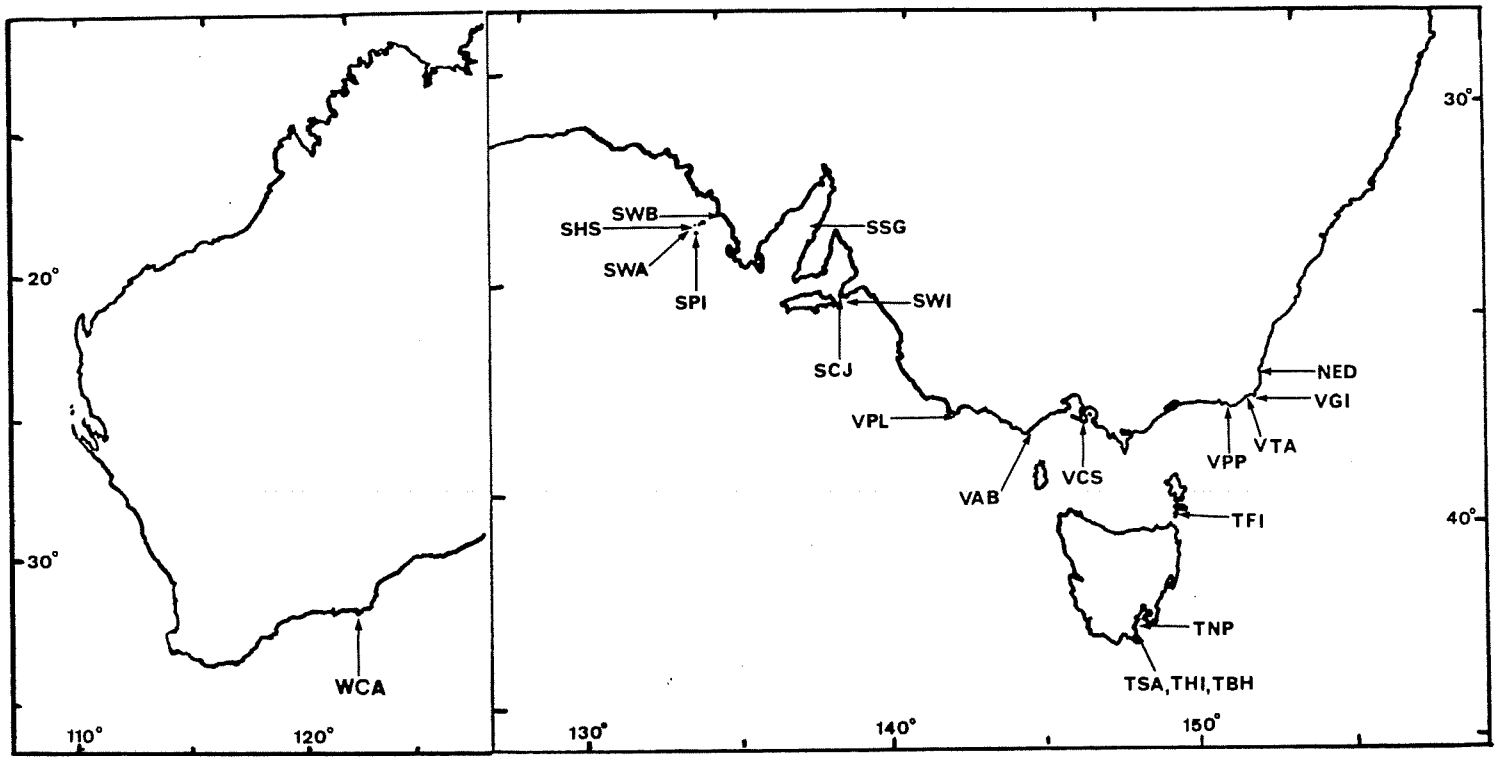

Figure 1. collection sites for *H. rubra* and *H. laevigata*. *H. rubra* (NED, VGI, VTA, VPP, VCS, VAB, VPL, TFI, TNP, TBH, THI, TSA, SWI, SCJ, SWB, SPI, WCA). *H. laevigata* (TFI, SWI, SCJ, SSG, SWB, SHS, SWA, SPI, WCA(sample not yet analysed), VPL(not yet sampled)).

Breeding populations of both species appear to be genetically large. Overall measures of genetic variation within populations (heterozygosities) are large: 0.14 for *H. rubra* and 0.195 for *H. laevigata*. Significant departures from random mating (Hardy-Weinberg proportions) within population samples were the result of heterozygote deficiences. However these deficienies were not found across all loci and are therefore not likely to be caused by inbreeding or a Wahlund effect (sample comprising two or more subpopulations).

* There is a further sample planned for *H. laevigata*.

Haliotis rubra

Genetic differences between populations of *H. rubra* are small and geographically cumulative but small scale genetic heterogeneity suggests that local populations may be predominantly recruited from local stock.

Across the broad geographic scale significant allele frequency differences were found for eight of the twelve polymorphic loci analysed. Examination of the relative contributions of population samples to overall heterogeneity shows that no population stands out as very genetically discrete. Rather, differences are small and geographically cumulative. This is shown by the relative positions of sample populations in the dendrogram based on genetic distance (Figure 2).

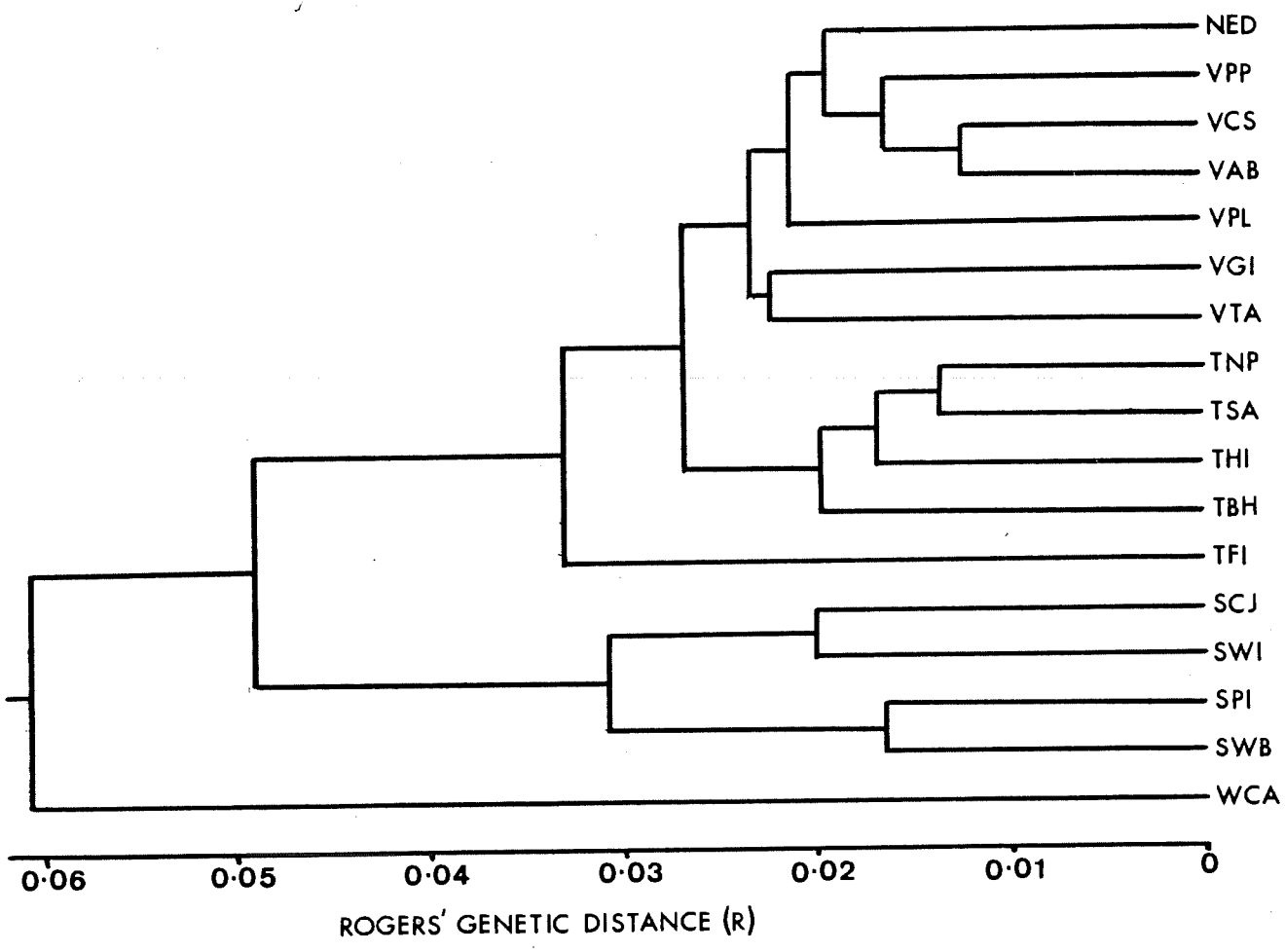

Figure 2. UPGMA dendrogram for seventeen populations of *H. rubra*. Genetic distance based on fifteen loci (twelve polymorphic, three monomorphic).

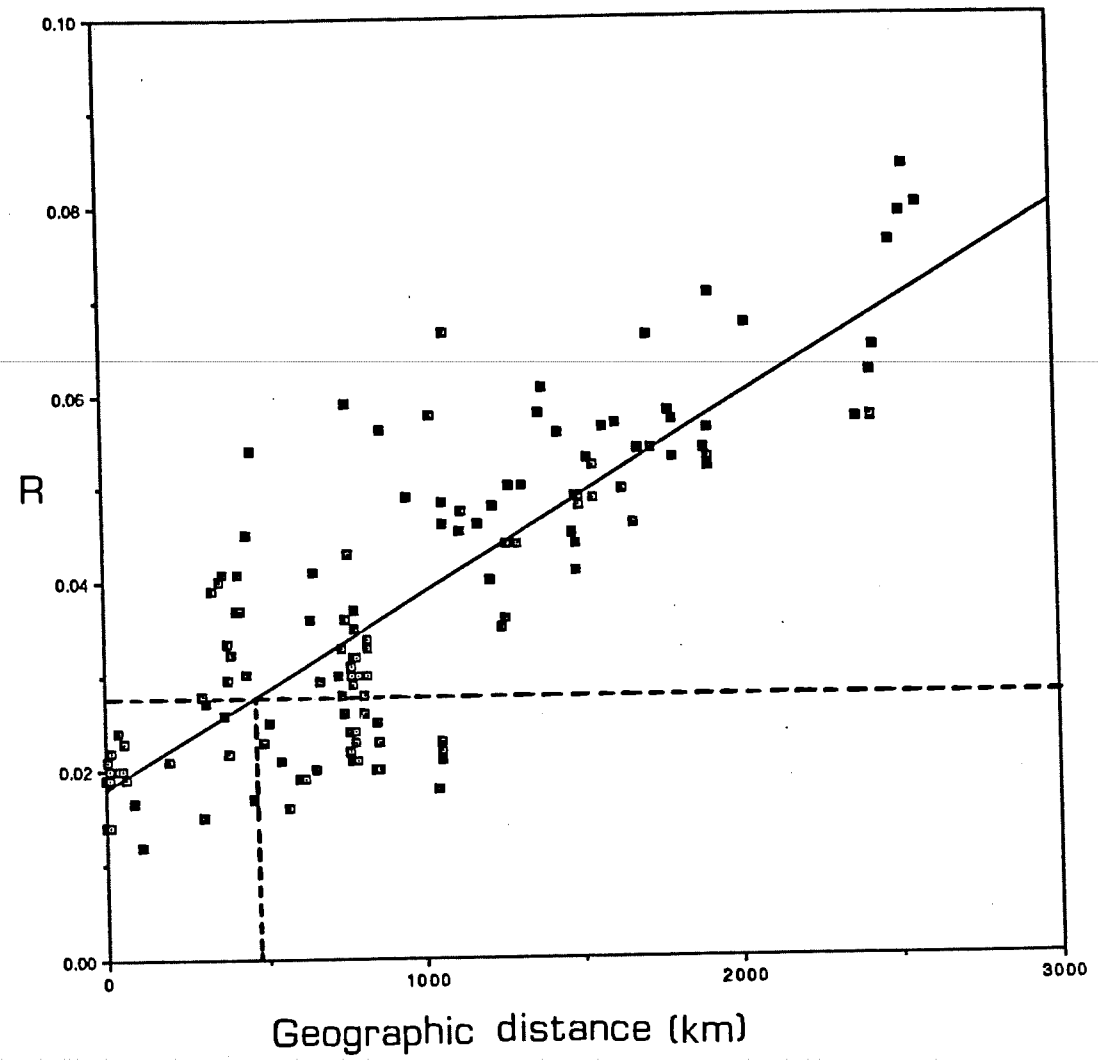

Figure 3. Regression of Rogers' genetic distance (R) on geographic distance for *H. rubra*. The solid line is the line of best fit. The dashed line indicates the average 'R' value found between six pairs of replicate samples.

Figure 3 shows the relationship between pairwise genetic distances between populations and geographic (shortest across water) distance. The correlation here is positive and significant ($r = 0.828$, $p < 0.01$, d.f.= 16). From this graph neighbourhood size (the region within which mating is effectively random) can be estimated (Richardson *et al.* 1986). The average geographical size of a neighbourhood is the X-coordinate of the regression of genetic distance (R) on geographic distance for the average 'R' value found between replicate sample sets. For *H.rubra* this is approximately 500 kilometres. **From a management viewpoint it seems reasonable that, for *H. rubra*, zones of about 500 kilometres of coastline could be recognised for the conservation of regional genepools.**

Analyses on a smaller geographic scale were carried out in Port Esperance, Tasmania. Three sites between 1 and 2.25 kilometres apart were selected: one site was an island, the others shore based. Significant differences in allele frequencies were found between sites for two gene loci. In both cases the island population contributed most to the heterogeneity. Even though populations have been large and interconnected during the species history **this observation suggests that for physically isolated reef and island populations local populations may be predominantly recruited from local stock.**

Haliotis laevigata

The population structure of *H. laevigata* appears to be different from that of *H. rubra*. However this is almost certainly partly due to incomplete sampling. The TFI sample stands out as very distinct (figure 4) but there are currently no samples between it and the other, all South Australian samples. These samples also show relatively large genetic distances from each other. This is likely to be because these populations are relatively isolated islands or reefs. Further sampling of connected coastline populations may reveal a geographically cumulative population structure similar to that of *H. rubra*.

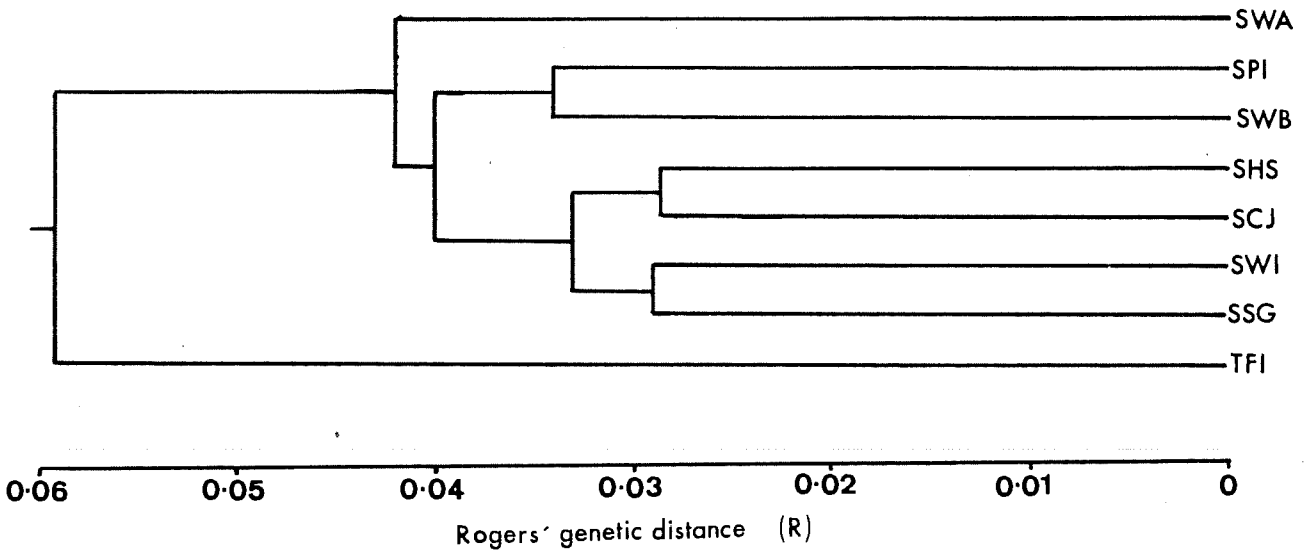

Figure 4. UPGMA dendrogram for eight populations of *H. laevigata*. Genetic distance based on fifteen loci (thirteen polymorphic, two monomorphic).

The relationship between pairwise genetic distance (R) and geographic distance is illustrated in figure 5. The correlation is positive and significant ($r = 0.805$, $P < 0.01$, $d.f. = 7$). The maximum 'R' found from the two sites where replicate samples were made estimated a neighbourhood size of zero kilometres. This essentially means that these populations of *H. laevigata* need to be regarded as separate genepools and management units.

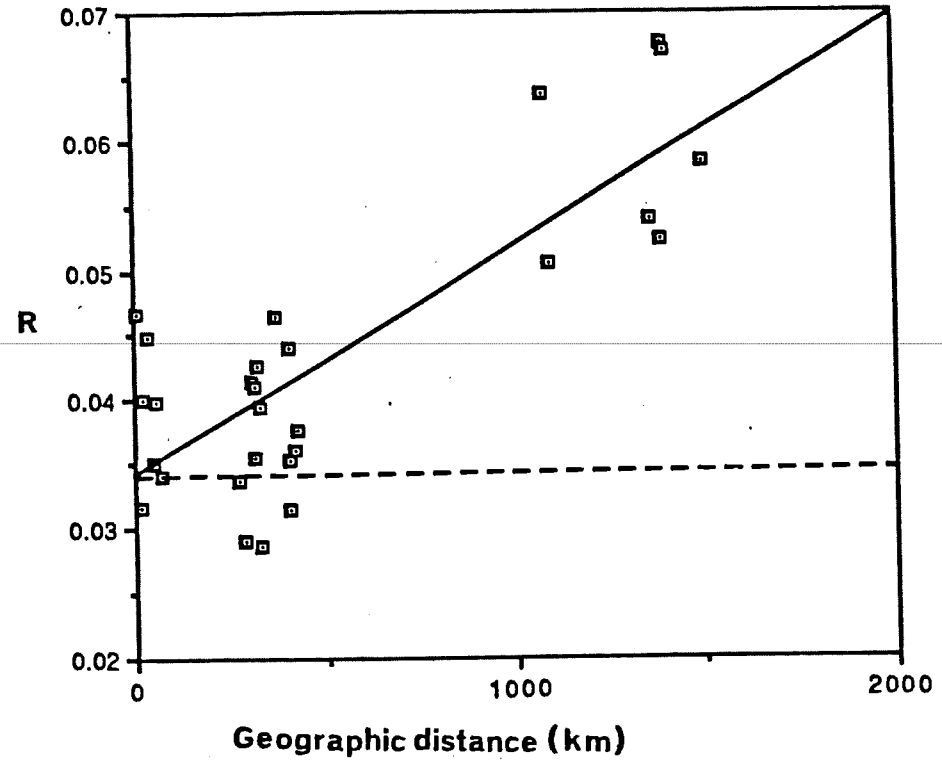

Figure 5. Regression of Rogers' genetic distance (R) on geographic distance for *H. laevigata*. the solid line is the line of best fit. The dashed line indicates the maximum 'R' value found between two pairs of replicate samples.

B. To determine whether and to what extent inter-breeding between blacklip and greenlip abalone occurs.

The existence of fixed allelic differences between *H. rubra* and *H. laevigata* at seven loci allows the identification of interspecies hybrids as first generation (F_1) crosses or as the products of backcrossing to either parent species. Of the seventeen morphologically intermediate individuals examined, eleven appeared to be F_1 hybrids i.e. they were heterozygous at all discriminating loci. Three individuals had *H. rubra* genotypes at at least one of these loci, and must have resulted from backcrosses of F_1 hybrids to *H. rubra*. The other three, for similar reasons were identified as backcrosses to *H. laevigata*.

Hybrids were found to be widely distributed with specimens coming from South Australia (SCJ), Victoria (VPL, VCS) and the Furneaux Islands (TFI); see figure 1. Relatively higher concentrations of hybrid individuals were found to occur at the Furneaux Islands and within these islands hybrids were only to be found at particular sites.

This work has led to additional research into natural and cultured hybrids by the Victorian Institute of Marine Sciences. The main aim of that project is to compare the performances of hybrid and parental species for aquaculture.

C. **To obtain information on the spatial and temporal distribution of genetic variation in blacklip and greenlip abalone which will be of vital importance in the development of future artificial culture and reef enhancement programs.**

Because natural populations have high heterozygosity levels it is likely that inbreeding would lead to low fitness and should be avoided in culture programs.

Moreover, information gained on the geographical distribution of genetic variation allows the recognition of regional genepools as management units.

The existence of genetically different populations from different regions suggests that for both species a wide range of potentially different stocks is available for aquaculture. This range should be fully explored to provide an optimum cultured genepool.

Any reef enhancement schemes will also need to take account of these findings.

D. **To provide the above information to fisheries managers and to liase with them to facilitate better assessments of stock/recruitment relationships, and hence sustainable yield in the major south -eastern abalone fisheries.**

The scientific staff of each of the southern states fishery departments involved in the management of abalone fisheries have made this project possible through assistance with collection of specimens and providing helpful advice and information. We have kept them up to date with our research by presenting our latest results at the annual Demersal Mollusc Research Group meetings.

In addition throughout the period of the project results have been presented at various conferences (Australian Marine Sciences Association, Genetics Society of Australia and the International Symposium on Abalone Biology, Fisheries and Culture). Results are currently being prepared for publication in the Proceedings of the First International Symposium on Abalone Biology, Fisheries and Culture (Brown and Murray 1990ab) and in appropriate scientific journals.

REFERENCES

Brown L.D. and N.D. Murray, 1990. Population genetics, gene flow, and stock structure in *Haliotis rubra* and *Haliotis laevigata*. In proceedings of the first Symposium on Abalone Biology, Fisheries and Culture. In press.

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