89/25

Stock Identification and Discrimination of Pilchards in Australian Waters, using Genetic Criteria.

by P. I. Dixon, L. J. Worland and B. H. Y. Chan,

University of New South Wales.

ACKNOWLEDGEMENTS

This project was financed by the Australian Government Department of Primary Industry's Fishing Industry Research and Development Council (FIRDC project No. 89/25).

We would like to extend special thanks to Dr W.J. Fletcher of the W.A. Marine Research Laboratories for organising the W.A. samples and the fishermen who collected them. We would also like thank the following people for providing samples: Dr C.M. MacDonald (Marine Science Laboratories, Queenscliff), the Mitchelson Brothers (Lakes Entrance), Dr B. Bruce (S.A. Department of Fisheries), Mr John Watson (Jervis Bay), Mr Jeff Cootes (Wooli) and Mr Augy Syahailatua (UNSW).

Mr Phillip England conducted the initial pilot study for this project.

EXECUTIVE SUMMARY

This study indicates that a series of contiguous, quasi-independent pilchard subpopulations, (quasi, because mixing occurs at some stages) inhabit Australian waters; a finding also supported by Blackburn (1951) and Syahailatua (1992). The subpopulations are temporally unstable and it is unclear how much mixing occurs during the pilchard life cycle. For example, two separate subpopulations were detected in King George Sound, WA, within a six week period.

The subpopulations can be grouped into regions as geographic stocks which have genetic integrity (with some intermixing) viz: the eastern, south-eastern, south-western, and western stocks. These stocks generally conformed with those outlined by Blackburn (1951) and Syahailatua (1992). Both authors contended that there was an additional subpopulation to the north of Jervis Bay. Deterioration of a sample from that area precluded us from testing that hypothesis but one sample collected from Jervis Bay suggested mixing of two or more subpopulations.

The samples of pilchards that we had from sites east of Esperance, WA, were from regions where only one spawning season per year has been documented. Those spawning seasons all vary in timing which would effectively act as a temporal isolation mechanism to interbreeding. There are two breeding seasons in most of southern Western Australia but no significant variation was detected between the summer and winter spawners. Thus, a more complex mechanism than temporal breeding isolation must operate to maintain the population structure. Homing to an individual's spawning place has been invoked for other Clupidae species and there is no evidence to dispute that homing occurs in the Australian pilchard.

It is difficult to effectively regulate individual subpopulations (ie: genetically distinct "stocks" in a management context) in a mixed-stock fishery, particularly when the degree of mixing is unknown at all life history stages. Blackburn (1951) discerned differential growth rates between stocks on the east coast so, it is clearly important to ensure that these subpopulations are protected using an appropriate management strategy.

CONTENTS

LIST OF TABLES PAGE

LIST OF FIGURES FACING PAGE

LIST OF APPENDICES ..... PAGE

INTRODUCTION

Australian pilchards, *Sardinops sagax neopilchardus*, are small, relatively short-lived fish that occur in large numbers, over a wide geographic range. They are distributed throughout coastal waters and in bays and inlets extending from Hervey Bay in Queensland, down to and around Tasmania, across Victoria, the Great Australian Bight (GAB) and up the west coast of Western Australia (WA) to Red Bluff (Fletcher 1990).

Despite the apparent abundance of this species, it had not been fished intensively until 1983. The national Australian pilchard catch currently exceeds 11 000 tonnes annually. The bulk of this catch is landed in Western Australia where the fishery has undergone steady and substantial expansion. The annual production for WA is approaching 10 000 tonnes (Fletcher *et al.* 1992). The WA fishery is concentrated around King George Sound/Albany, Bremer Bay, Esperance, Dunsborough and Fremantle. The resource is known to extend eastwards into the Bight and beyond.

In South Australia, the catch is small and fluctuates annually. Most pilchards are used for tuna and snapper bait and to supply local tuna farms which also use imported frozen pilchards from WA (B. Bruce pers. comm.).

Until the early 1980's, a substantial fishery for pilchards (and anchovies) existed in the Lakes Entrance area of Victoria. Currently, the annual production is approximately 1 000-1 500 tonnes from Port Phillip Bay and 1 000 tonnes from Lakes Entrance. These pilchards are mainly used for pet food and snapper bait. Controversy in the early 1990's about starving fairy penguins (*Eudyptula minor*) rekindled debate about whether or not pilchards were being over exploited (M. MacDonald pers. comm.). Population studies of pilchards and anchovies are planned for Victoria (but are awaiting funding).

In New South Wales, there is a small fishery for pilchards; activity is restricted to Jervis Bay, Wooli and Two Fold Bay (Eden). The annual catch is between 140-300 tonnes (Syahailatua 1992). Since the collapse of the Southern Blue Fin Tuna (SBT) fishery in NSW, the catch is mainly sold for human consumption.

Small catches of pilchards have been made in Tasmanian waters but there is no existing fishery. Similarly in Queensland, there is no targeted fishery, but pilchards are known to be numerous in these waters from time to time.

Pilchards and related species have been the subject of sporadic biological research since the 1930's. Blackburn (1950, 1951) studied pilchards extensively during the 1940's and '50s. Subsequently, he reported three major groups in Australian waters; within each group there was further heterogeneity. Those conclusions were based on differences in breeding time, age at first maturity, scale size, vertebral counts and abundance fluctuations. Blackburn contended that independent or quasi-independent stocks with small ranges were likely, but he had insufficient data to attempt to delineate them. Syahailatua's (1992) study of Australian pilchards, using discriminant analyses of morphometric and meristic characters, indicated that there are six groups of pilchards within Australian waters.

Dredge (1969) carried out a preliminary genetic study on pilchards from South Australian waters. He studied only two enzymes from heart tissue; viz: lactate dehydrogenase and an unspecified esterase. Both enzymes were polymorphic and differences were found between each of the three sites sampled (Coffins Bay, Cape Jervis and Port Adelaide). This pioneering work, although very limited, also supported the idea of small subpopulations.

Expansion of the fishery in WA, during the early 1980's, prompted research into Australian pilchard resources. Those findings were presented in June 1988 (SCP 1988). In addition, the WA Fisheries Research Division has undertaken comprehensive biological research on pilchards. These findings are presented in Fletcher *et al.* (1992,) Fletcher (1992) and Fletcher and Tregonning (1993).

The use of the terms population, subpopulation and stock varies between authors. In this study, population refers to the whole Australian pilchard resource and a subpopulation is a genetically distinct group within that population. Stock is a broader term. Syahailatua (1992) for example, referred to distinct stocks without knowledge of the underlying genetic structure. A stock is also a unit within the population defined for geographical or management convenience which may, or may not, contain genetically discrete units. Where other authors have defined "stocks", we have retained that term but they do not necessarily refer to distinct subpopulations.

Pilchards from Jervis Bay, on the east coast of Australia, spawn from mid autumn to spring (Blackburn 1951, Joseph 1981, and Syahailatua 1992); in spring to early summer around Lakes Entrance; late spring and summer in Port Phillip Bay and summer to autumn in South Australia (Blackburn 1951). Two distinct spawning seasons have been reported for some regions of the west Australian coast (Fletcher 1990). However, it is not known whether all fish, can and do, spawn biannually, or whether there are separate groups of winter and summer spawners. There is a major

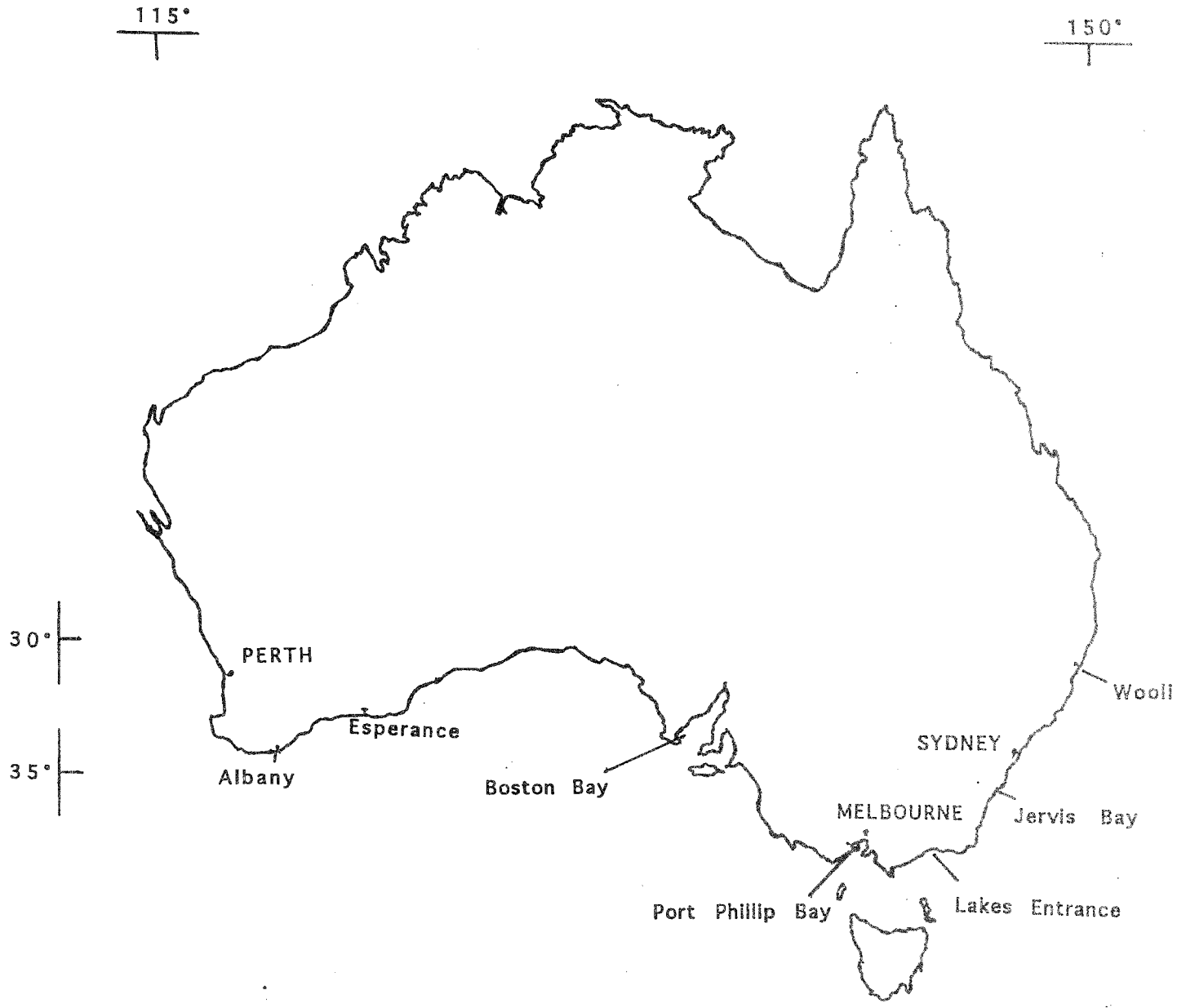


Figure 1: General collection sites of *S. neopilchardus* from Australian waters.

spawning period in July off Albany and Bremer Bay, WA, and there is a degree of larval movement between these two regions, but there is some possibility that the adults in these respective areas function as independent units (Fletcher *et al.* 1992). Little is known about the summer WA spawning season when catches are traditionally poor. In the Atlantic herring, *Clupea harengus harengus*, Kornfield *et al.* (1982) found genetic evidence of discrete spring and fall spawning subpopulations, where mixing occurs at other life history stages.

Groups that are partly or wholly isolated as breeders are expected to differ in morphological or physiological characters (Blackburn 1951). This project investigates the genetic population structure of *S. s. neopilchardus* within Australian waters. The main objective is to determine whether they belong to one large interbreeding population or, whether two or more genetically discrete subpopulations exist. In the event that heterogeneity is identified within the Australian pilchard population, attempts will be made to delineate subpopulations. Isolated gene pools can respond independently to ecological variation or fishing pressure thus, once spawning populations of migratory species have been characterized, fisheries managers can address questions on mixed stocks (Chapman and Brown 1991).

2.0 MATERIALS AND METHODS

2.1 Specimen Collection for Pilot Study

For initial enzyme screening in the pilot study, approximately 100 frozen specimens were collected from Bremer Bay, King George Sound and Two People Bay in Western Australia, and from Jervis Bay in New South Wales. A chilled sample was also obtained from Wooli, NSW. All samples were collected using purse seine nets.

2.2 Specimen Collection for Population Study

Appendix 1 presents the collection site and date, number of individuals, sex ratio, size range and breeding status of each sample investigated in this study. Spawning condition was only assessed macroscopically, and because specimens needed to be kept frozen, it was not possible to test for "ripe" individuals in the usual manner (see Appendix 1.2 and Baker 1972).

For the population study of *S. s. neopilchardus*, specimens were collected from several Australian states (Figure 1). The bulk of the samples were collected from six regions of Western Australia (Figure 2). Dr W. J Fletcher (Western Australian Fisheries Research Division) coordinated the collection of pilchards by commercial fishermen.

These specimens were collected as part of a routine sampling program at three month intervals throughout 1990 and 1991 (ie: Dec-Jan, April-June, August-September).

Kornfield *et al.* (1982) note that only fish in spawning condition should be examined in geographical studies. However, for management purposes the genetic structure of breeding units must be related to those found in the population at other times of the year hence, the August-September collecting period.

For some WA sites we received several small samples of pilchards collected on separate days. In order to meet sample size requirements for statistical purposes, samples that did not differ in gonad maturity were pooled (see Appendix 1).

From New South Wales waters, pilchards were collected from Wooli in April 1990 with the assistance of commercial fishermen. However, on arrival the chilled specimens were in poor condition for electrophoretic purposes. We were unable to obtain subsequent samples from this location. Pilchards were also collected from Jervis Bay in February 1991 and March 1992.

With the assistance of Dr Murray MacDonald (Marine Science Laboratories) pilchards were obtained from the Geelong arm of Port Phillip Bay, Victoria, in February 1991 and from the top end of Port Phillip Bay in March 1992. Specimens were collected from Lakes Entrance by the Mitchelson fishermen in August 1991. These fish varied in size classes from 80.1 mm to 190.3 mm L.C.F. This range would include individuals 1 to 6 year old (Blackburn 1950). There were insufficient numbers of each size class to analyse by age, so only the middle range of individuals from 127.4 mm to 149.6 mm were included. This range encompasses the 3-5 year olds (Blackburn 1950).

From South Australia pilchards were collected from Boston Bay, Port Lincoln in April 1992. They were kept alive in a bait tank and then frozen live four days later. An additional small sample of pilchards, presumably *S. ocellata,* from South Africa was also obtained.

All fish, except the Wooli sample, were frozen live and then transported to the laboratory, either on dry ice, or by refrigerated trucks. Upon arrival, all specimens were stored whole at -20^{0}C, until required.

2.3 Preparation of Starch Gel

Horizontal starch gels were made from 11% (w/v) Electrostarch, batch number 89, with a range of buffers (see Appendix 2.1). Starch (33 g) was suspended in 1/3 of 300 ml of gel buffer, put on a stirrer while the remaining 2/3 of buffer was heated to

boiling point in a microwave oven (1 minute per 100 ml). The stirring solution was then transferred to a pre-heated hot-plate, and the remaining solution was quickly added. It was kept stirring until the volume reduced and the solution was clear with bubbles rising from the bottom of the flask. This solution was then degassed using a Gelman "Little Giant" vacuum pump and poured into a square perspex mould (Dimensions: 300 ml 19.5 x 19.5 x.5 cm; or 500 ml: 19.5 x 19.5 x 1 cm).

Once poured, the gel was left to cool at room temperature for at least 1 hour, covered with plastic food wrap to prevent desiccation and stored overnight in a refrigerator.

2.4 Tissue Preparation

Fish were partially thawed and particular tissues were removed for electrophoresis. For the pilot study, liver, heart and white skeletal muscle were removed and screened for tissue specificity of enzyme loci. For the main population study, liver was the only tissue used for routine screening. All tissue samples were stored in 1.5 ml eppendorf tubes for immediate use and in 1.8 ml nunc cryotubes as backup tissue. These were all stored at -80^{o}C until required for electrophoresis. All fish specimens were then stored at -20^{o}C until they were measured (standard fork length, L.C.F.), sexed and gonad phase noted.

Tissue samples were partially thawed and homogenized with an equal volume of cold homogenizing buffer (see Appendix 2.2) using a perspex rod[1]. Tissue samples were kept in small ice-filled insulated containers during homogenization. The slurry was then centrifuged using an Heraeus Sepatech 17RS Biofuge at 4^{o}C, at 5000 rpm for 10 minutes. This process separates cellular debris from the supernatant containing proteins in solution.

2.5 Electrophoresis

2.5.1 Pilot Study

Six different buffer systems viz: Cam pH 6.1, TBE pH 9.1, TC pH 6.8, TC pH 5.8, TM pH 7.8 and Poulik, were investigated in the pilot study. The recipes of the buffers, stain buffers and stock solutions used throughout the study are given in Appendix 2.1-2.6). Liver, heart and white skeletal muscle were trialed with the buffers. Initially, 1-3 buffers were tried and if good activity was apparent, other buffers were then tried to ascertain which buffer system yielded the best activity and resolution for polymorphic loci.

After homogenisation and centrifugation, the supernatant was absorbed onto a filter paper wick (Whatman #3 filter paper, 5 x 2 mm) and using jeweller's forceps, placed onto the edge of a cut starch gel 5 cm from the cathodal end of the gel. A wick blotted in bromophenol blue solution was placed on the first lane on the left-hand side of the gel and after the last homogenate on the right-hand side.

Loaded gels were placed in electrophoresis tanks with the appropriate electrode buffer. A direct current was applied across the gel using Pharmacia power packs according to the conditions prescribed for the buffer as given in Appendix 2.1.

Gels were run until the tracking dye had migrated 5-7 cm from the origin (after 3h)[2]. Gels were then sliced three times. Each slice was removed using a pre-cut plastic sheet and placed in a plastic tray. The gels were stained using methods modified from Shaw and Prasard (1970) and Harris and Hopkinson (1976) (Appendix 2.5)[3]. Gels with activity were photographed and scored.

The zymograms were scored by tissue type for amount of activity, resolution, presumed number of loci, structure and whether they appeared polymorphic (see Appendix 3.2).

2.5.2 Population Study

The laboratory techniques for the electrophoresis population study follow the same technical methods as described for the pilot study. Up to 100 individuals per sample were electrophoresed and scored for polymorphic loci. The nomenclature of loci follows the recommendations of Shaklee *et al.* (1990a). Multiple loci were numbered sequentially with the most anodal (fastest migrating) locus designated as 1, eg: *EST-1* migrates faster than *EST-2* (Richardson *et al.* 1986).

Alleles were assigned arbitrary values with the most common allele nominated as *100*, faster alleles were assigned numbers in increasing units of 10 in preference to using

---

1. Pyrodoxil 5' Phosphate was added to the homogenising solution (see Shaklee *et al.* 1990b).

2. The peptidase loci were subject to diffusion (and appeared fuzzy) if the standard running time (ie. 3 h/300 ml gel) was not strictly adhered to. The other enzymes were more flexible in this regard.

3. *AAT** overstained very quickly which also made scoring difficult. Trying to freeze slices, for approximately ten minutes, before staining, did not improve the stain. Instead, slices for *AAT** stains were left in a refrigerator for at least ten minutes before staining. *AAT** resolved well on both TC 5.8 and TM 7.8 so, both were scored and compared. Any individuals that differed between slices were re-run for clarification and compared to their photographs.

specific migratory distances. That is, the next anodal allele was *110* and so on. Conversely, slower alleles were typed in decreasing values of 10. This allowed for the inclusion of any previously undetected alleles. Such alleles were assigned values according to mobility in relation to other alleles. After all samples had been screened, these numbers were converted to alphabetical letters for analytical convenience (the most common allele *100* was designated as *c*).

Gels in the population study were usually run with 24 unscreened individuals with the first individual repeated after the 24th individual. A bromophenol blue wick was placed at the beginning of the gel and after the repeated first individual (ie. in lane 26). For consistency in typing alleles between gels, each gel was run with two standards of known genotype. For consistency in typing on the same gel, the distance between the origin and each allele was measured using dividers.

2.6 Data Analysis

The genotype for each individual, at each of the six loci, was recorded and analysed using the BIOSYS-1 program by Swofford and Selander (1989). Initially, allele frequencies were calculated to determine genetic diversity. Each sample was then tested for conformity to Hardy Weinberg equilibrium to ensure that samples were homogeneous.

Analyses testing for temporal variation were conducted using a contingency chi-square statistic in the BIOSYS-1 program. Homogeneous samples collected from the same site which were not significantly different ($p>0.05$) were pooled together and retested for conformity to Hardy Weinberg equilibrium (see Shaklee *et al.* 1990b).

Kornfield *et al.* (1982) explain that it is critical that only spawning fish be examined to delineate geographical relationships, because mixing occurs during non-spawning periods. The spatial analyses were therefore undertaken on homogeneous samples of spawners where possible.

We emphasize a temporal rather than a spatial analysis of our data because inferences about underlying population structure deduced from particular patterns of spatial variation may be inaccurate if such patterns vary markedly over time (Kornfield *et al.* 1982).

Length frequency histograms were plotted for each sample and are presented in Appendix 4.0.

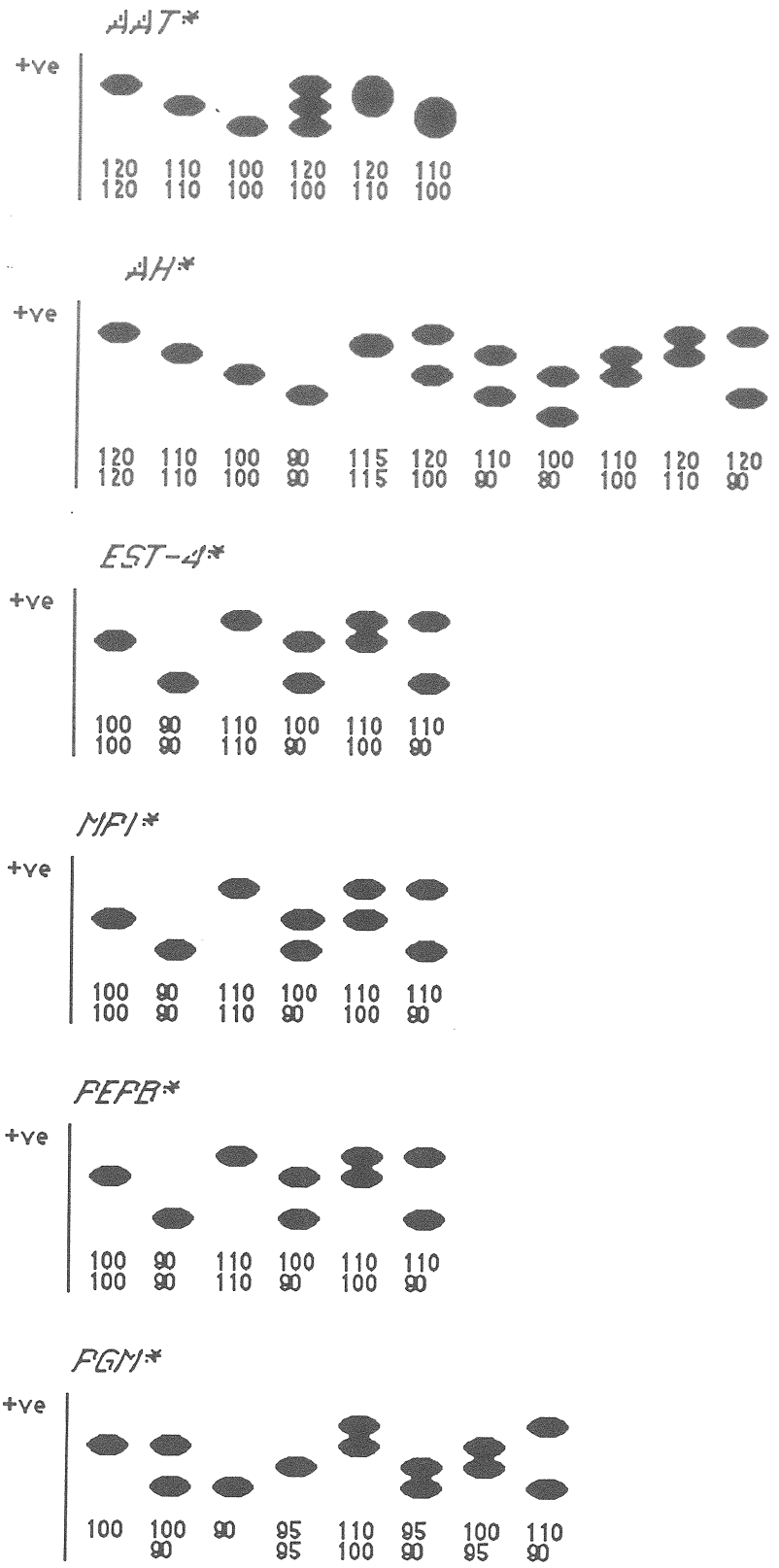


Figure 3: Observed banding appears and designated genotypes for the six enzymes screened in the population study.

3.0 RESULTS

3.1 Results of Pilot Study

The pilot study included samples collected from three different locations in WA and from Wooli and Jervis Bay in NSW. The Wooli sample was not transported frozen and much of the enzyme activity was lost. Because this sample yielded inconsistent and inconclusive results, it was excluded from further consideration.

Forty-eight enzymes were screened in liver, heart and muscle tissue on six different buffer systems (see Appendix 3.1). Ten of those enzymes revealed no activity and 13 presumed loci were unscorable from eleven enzymes. The remaining 27 enzymes represented 31 presumed loci. This information is summarised in Appendix 3.2.

Of those 31 presumed loci, 7 were polymorphic with sufficient resolution for scoring. The polymorphic loci were Aspartate aminotransferase (*AAT**, E.C 2.6.1.1), Aconitate hydratase (*AH**, E.C. 4.2.1.3), Esterase-4 (*EST-4**, 3.1.1.1), Mannose phosphate isomerase (*MPI**, E.C. 5.3.1.8), Peptidase-B (leu-gly-gly) (*PEPB**, E.C. 3.4.11 or 3.4.13.9) and Phosphoglucomutase (*PGM**, E.C. 5.4.2.2). The other polymorphic locus, Peptidase (leu-leu-leu) was also scorable but the same individuals for *PEPB** were polymorphic so it was excluded from the routine scoring. See Figure 3 for zymograph illustrations and interpretation of the routinely scored loci. Gene nomenclature follows the recommendations of Shaklee *et al.* (1990a).

The acceptable level of polymorphism was set at the .95 criterion however, during routine screening there were a few samples which had *MPI** frequencies between the .95 and .99 criteria (see Appendix 6.2).

3.2 Results of Population Study

Initially, all samples were analysed within their respective collection period ie. May 1990, September 1990, Summer 1990-91, May 1991 and Summer 1991-92. However, most loci showed significant deviation ($p<0.01$) from Hardy Weinberg equilibrium. This was probably due to the occurrence of 'rare alleles' at most loci. We were unable to discern any pattern in the frequencies of rare alleles (see Appendix 5) thus, it was considered unlikely that pooling would mask any significant differences between samples. Samples within the same collection period from the same site which were not significantly different ($p>0.01$) were pooled. This enhanced the number of individuals per sample for statistical purposes (see Appendix 1). Appendix 6.1

provides the rationale for pooling alleles at each locus. The pooled allele frequencies for each initial sample are given in Appendix 6.2.

Each sample was tested for conformity to Hardy Weinberg equilibrium (Appendix 7.1). Generally, the *AAT** locus was significantly out of equilibrium ($p<0.05$). This locus stained very quickly, despite preventative measures, and heterozygotes may have been scored too conservatively, however, the frequencies were consistent throughout. The scoring of all loci that were out of equilibrium were rechecked with the negatives of their respective zymograms. All appeared to have been scored correctly so no changes were made.

The *MPI** and *PEPB** loci often deviated significantly from Hardy Weinberg equilibrium. This was usually due to low expected values for the uncommon homozygote despite pooling of rare alleles, so samples were not classed as heterogeneous in those cases.

Most samples were within a similar size range (see Appendix 4.0) and were all approximately 3-5 year olds (Fletcher 1990). Sample sizes were too small to be separated into single year classes. Many of the pilchard samples were dominated by a particular sex. To ensure that none of the loci were sex linked, the second Port Phillip Bay sample (PPB2) which had an even ratio of each sex, was divided into males and females and tested for fit to Hardy Weinberg equilibrium (see Appendix 7.2) then compared using a contingency chi-square test. Both sexes were in Hardy Weinberg equilibrium ($p>0.05$) except at the *AAT** locus (see above). The male and female samples were not significantly different at any locus ($p>0.05$) (see Appendix 7.2.3), so we conclude that the six loci used in the following analyses are not sex linked.

There was a large range in *EST-4** frequencies (see Appendix 6.2), so the most extreme ones were rechecked with photographs of the appropriate zymograms but they appeared to be correct. The frequencies were then arbitrarily categorised and the samples were sorted according to frequency and season (see Appendix 8.0). No pattern in frequency for site or season was apparent.

3.2.1 Temporal Variation between Samples.

For most WA sites we had multiple samples collected over a two year period. This enabled us to examine temporal variation between samples. We tested that there was no temporal variation within sites:

a) within years for winter and summer spawners, separately;
b) between spawners of the same breeding season between years;
c) between non-spawners, either within or between years;

d) between summer and winter breeding seasons, either within or between years; and
e) between spawners and non-spawners.

For each site these comparisons were performed in a hierarchical manner using the pooled allele frequencies. The temporal variation results are presented in the following.

SITE: King George Sound (KGS), WA

Among the seven samples collected from KGS, only samples KGS1, KGS5 and KGS6 were in Hardy Weinberg equilibrium (p>0.05) and thus considered to be homogeneous (Appendix 7.1.1).

Table 1 presents the levels of significance for the temporal variation comparisons. The first set of comparisons address the hypotheses as described at the beginning of this section. The latter half of Table 1 examines the hypothesis that there is no variation between samples dominated by a particular sex.

Table 1: Levels of significance of contingency chi-square comparison for each locus betweenKing George Sound, WA samples (Appendix 1). Abbreviations: W. = winter, S. = summer (S '90= Dec 90-Feb 91, S '91=Nov91-Jan92), Sp=spring, Au= autumn, **v.** =compared to, M=male dominated, F=female dominated, sp=spawners, non=nonspawners. a, b, c, and e refer to section 3.2.1 hypotheses.

COMPARISON	BREEDING STATUS	SEASON, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
a) KGS5 **v.** 6	spawners	W'91, W'91	.110	.015	.018	.770	.830	.060
b) KGS2 **v.** 5	"	W'90, W'91	.309	.164	.087	.202	.956	.948
b) KGS2 **v.** 6	"	W'90, W'91	.749	.488	.740	.099	.804	.100
b) KGS4 **v.** 7	spawners	S '90, S '91	.640	.010	.000	.510	.100	.135
c) KGS1 **v.** 3	nonspwners	Sp'90, Au'90	.110	.145	.044	.380	.090	.520
e) KGS5 **v.** 1	sp, non	W'91, Au'90	.494	.075	.078	.584	.140	.547
e) KGS6 **v.** 1	sp, non (M)	W'91, Au'90	.043	.910	.940	.409	.174	.370
KGS6 **v.** 7	spawners (M)	W'91, S'91	.295	.610	.075	.700	.811	.018
KGS4 **v.** 5	spawners (F)	S'90, W'91	.926	.269	.629	.495	.704	.247

The Winter '91 samples (KGS5 and 6) which were collected from Albany within a six week period and were rated as pre-spawners and mixed spawners respectively (Appendix 1), were significantly different (p<0.05) at the *AH** and *EST-4** loci, and close to significance at the *PGM** locus. When KGS5 and KGS6 were individually

compared to KGS2, a sample of winter spawners from the previous year, neither comparison was significantly different at any locus ($p>0.05$) (Table 1).

By arbitrarily ranking the frequencies of *AH** and *PGM** as low, medium and high for these three samples, the KGS2 frequencies for both loci were intermediate between the winter spawners of the following year (Table 2). It is also apparent that the winter '91 samples differed in sex composition whereas KGS2 had a 1:1 ratio of females to males. Furthermore, when the winter spawning samples were pooled and tested for Hardy Weinberg equilibrium, there was a significant heterozygote deficiency at the *AAT**, *AH**, *EST-4** and *MPI** loci ($P<0.01$) (Appendix 7.3.1). *PEPB** and *PGM**, although not significant ($p>0.05$), also showed heterozygote deficits. Hence, it was not possible to pool these sample as winter spawners and they were kept separate for subsequent comparisons.

The summer spawners (KGS4 and 7) were also significantly different at the *AH** and *EST-4** loci ($p<0.05$) (Table 1). Both samples did not fit Hardy Weinberg equilibrium expectations at the *AH** and *PGM** loci so, mixing of subpopulations within KGS appears to be consistent between those summer seasons (Appendix 7.1). The summer spawners were similar to the winter spawners in that there was a difference in the dominance of sexes and the *AH** locus had low ranking for the common allele in the female dominated sample (Table 2) however, *PGM** was not significant ($p>0.05$) (Table 1).

As there was heterogeneity within the samples from the winter and summer breeding seasons, it was not possible to pool and collectively compare them as winter and summer spawners.

The non-spawners collected in Autumn and Spring of 1990 (KGS1 and 3) were not significantly different ($p>0.05$) except at the *EST-4** locus ($p<0.05$) (Table 1). Samples cannot be regarded as different subpopulations on the basis of the *EST-4** locus alone, as it may not be solely under genetic control (see section 4.1). KGS3 was unlikely to be a homogeneous sample and when those samples were pooled, they were significantly different from Hardy Weinberg equilibrium expectations (Appendix 7.3.1).

There was no significant difference between homogeneous samples of spawners and non-spawners at any locus except between the KGS6 and KGS1 male dominated samples at the *AAT** locus. Comparisons between the KGS samples then focused on temporal variation and the predominant sex of the sample.

As mentioned in the above, the male dominated spawners and non-spawners, KGS1 and 6, were not significantly different ($p>0.05$) except at the *AAT** locus ($p<0.05$). The male dominated spawning samples collected in winter and summer of 1991 from KGS (6 and 7) were significantly different at the *PGM** locus ($p<0.05$) (Table 1). KGS female spawners that were collected in different seasons and years were not significantly different at any locus ($P>0.05$) but KGS4 was considered to be a mixed sample so these samples could not be pooled. These differences indicate that temporal variation exists between male dominated samples but not between female dominated samples from King George Sound.

Table 2: *AH** and *PGM** allele frequency rankings (high (h), medium (m) and low (l)) and sex ratios of grouped samples. *denotes heterogeneous samples.
The arbitrary frequency levels for *AH** are: h=>.580, m=.560-.579 and l=<.559
*PGM** are: h=>.794, m=.771-.793 and l=<.770.

SEASON	SAMPLE	Allele Frequency *AH**	*PGM**	Sex Ratio (F:M)
winter (spawners)	KGS6	.614 (h)	.710 (l)	0.88:1
	KGS2	.574 (m)	.793 (m)	1.00:1
	KGS5	.488 (l)	.796 (h)	4.00:1
summer(spwnrs)	KGS7*	.587 (h)	.809 (h)	0.83:1
	KGS4*	.420 (l)	.737 (l)(ns)	2.10:1
	ESP2+6	.596 (h)	.765 (l)	1.40:1
	ESP4+5	.500 (l)	.845 (h)	4.50:1
summer (nonsp)	JB1	.616 (h)	.753 (l)	1.69:1
	JB2*	.516 (l)	.729 (l)	0.68:1
	BB1+3	.563 (m)	.698 (l)	3.33:1
	BB2	.551 (l)	.810 (h)	1.40:1

SITE: Esperance (ESP), WA

ESP1 a sample of winter spawners (predominantly female) was significantly out of Hardy Weinberg equilibrium at the *AAT**, *AH** and *MPI** loci ($P<0.05$) (Appendix 7.1.2). The other 3 loci were close viz: *EST-4** ($p=0.08$), *PEPB** ($p=0.54$) and *PGM** ($p=0.06$). All differences were due to heterozygote deficits. It was therefore a heterogeneous sample. ESP3 a sample of juveniles fitted Hardy Weinberg expectations at all loci ($p>0.05$). The other samples from Esperance were generally within equilibrium ($p>0.05$) (see Appendix 7.1.2). Comparisons were then made between samples as described in section 3.2.1 and between Esperance adults and juveniles (Table 3).

Table 3: Levels of significance of contingency chi-square tests between Esperance, WA samples. See Table 1 for abbreviations. jvnls=juveniles.

COMPARISON	BREEDING STATUS	SEASON, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
a)ESP4 v. 5	spawners	W'91, W'91	.605	.476	.608	.005	.499	.543
b)ESP4+5 v.1 (no summer spawners)	spawners	W'91, W'90	.355	.221	.030	.048	.440	.069
c)ESP2 v. 6	non-sp	S'90, S'91	.922	.597	.003	.133	.692	.947
e)ESP2+6v. 4+5	sp, nonsp	W'91,S'90'91	.402	.070	.884	.251	.289	.058
ESP3 v. 4+5	jvnls,sp	W'91,W'91	.085	.114	.635	.117	.672	.001
ESP3 v. 2+6	jvnls,nonsp	S'90'91,W'91	.063	.768	.009	.163	.896	.130

The Esperance winter spawners collected in the same season and year were not significantly different ($p>0.05$) except at the *MPI** locus. *MPI** was not very polymorphic (see section 3.1) and is therefore sensitive to the occurrence of rare homozygotes. As pooled samples they fitted Hardy Weinberg equilibrium expectations (Appendix 7.3.2). These samples were considered as one for subsequent comparisons. ESP1 was not pooled with the winter '91 sample as it did not initially fit Hardy Weinberg equilibrium expectations.

The non-spawners collected from Esperance in summer (ESP2 and 6) over two consecutive years were not significantly different ($p>0.05$) except at the *EST-4** locus ($p<0.05$). This locus is not reliable without other significant loci to distinguish between subpopulations (see section 4.1). When these samples were pooled and tested for fit to Hardy Weinberg equilibrium (Appendix 7.3) only *AAT** and *MPI** deviated significantly ($p<0.05$). The summer non-spawners were pooled for later comparisons since *AAT** is often out of equilibrium and *MPI** due to a low expected value (see Appendix 7.1.2).

The winter and summer adults (spawners and non-spawners respectively) were not significantly different at any loci ($p>0.05$). However, the *AH** and *PGM** loci were close (see Table 3) so they were left as separate groups.

The winter juvenile sample was significantly different to the winter '91 spawners at the *PGM** locus ($P<0.01$). *PGM** was a reliable locus so it is unlikely that the winter '91 spawners were the source of the winter '91 juveniles. However, the juveniles were only significantly different to the Esperance summer non-spawners at the *EST-4** locus (this locus may change ontogenetically, or with environmental changes) but as the other loci did not differ they are not considered to be different. Thus, these adults could have been the source of the Esperance juveniles.

The *AH** and *PGM** loci in the Esperance samples varied in a similar manner to the KGS winter samples, particularly the predominantly female samples (Table 2).

SITE: Bremer Bay (BB), WA

Samples BB1, BB2 and BB3 were in Hardy Weinberg equilibrium ($p>0.05$) except at the *AAT** locus ($p<0.05$) where there was a large heterozygote deficiency (Appendix 7.1.3). In view of *AAT** consistently being out of equilibrium these samples were considered to be homogeneous. Sample BB4 was a heterogenous sample as it was significantly ($p<0.05$) out of Hardy Weinberg equilibrium at *AAT**, *AH** and *EST-4**, again there were large heterozygote deficits.

Table 4 presents comparisons between Bremer Bay samples following the hierarchy of hypotheses described at the beginning of this section.

Table 4: Levels of significance of contingency chi-square comparisons between Bremer Bay, WA samples. See above for abbreviations, "non-pre" (see Appendix 1)

COMPARISON	BREEDING STATUS	SEASON, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
b) BB1 v. 4	spawners	W'90, W'91	.891	.057	.001	.505	.613	.185
d) BB1 v. 3	sp., non-pre	W'90, S'90	.688	.264	.003	.136	.663	.721
e) BB1+3 v. 2	sp., non	W+S'90,Sp'90	.852	.804	.000	.399	.575	.033

The winter spawners collected in consecutive years from Bremer Bay were significantly different at the *EST-4** locus ($p<0.01$) and were close to significance at the *AH** locus. Furthermore, BB4 was a heterogeneous sample so they were not pooled.

Bremer Bay winter and summer spawners (BB1 and BB3) were not significantly different at any locus ($p>0.05$) except *EST-4**. These samples were pooled and fitted Hardy Weinberg equilibrium expectations except at *AAT** ($p<0.05$) (Appendix 7.3.2) so were compared to other samples as one.

The summer and winter spawners compared to the non-spawners collected from Bremer Bay were significantly different at the *PGM** and *EST-4** loci ($p<0.05$). *PGM** is reliable and indicates that the non-spawners were from a different recruitment source. The more predominantly female samples showed a different trend in the high and low rankings of the *AH** and *PGM** loci compared to KGS and ESP (Table 2).

There is no evidence to suggest that summer and winter spawners are different subpopulations from the results in Table 4, but there is some evidence of different subpopulations in the Bremer Bay area between breeding seasons.

SITE: Torbay (TOR), WA

The Torbay samples fitted Hardy Weinberg equilibrium expectations with the usual exception of *AAT**. TOR2 and TOR4 samples were out of equilibrium at the *PGM** and *AH** loci respectively, but it is unclear whether they were is due to chance, as other loci were in equilibrium (see Appendix 7.1.4). The contingency chi-square results testing for temporal variation between Torbay samples are given in Table 5.

Table 5: Levels of significance of contingency chi-square comparisons for Torbay, WA samples. See above for abbreviations.

COMPARISON	BREEDING STATUS	SEASON, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
b)TOR1 **v.** 4	spawners	W'90, W'91	.664	.386	.000	.336	.751	.163
d)TOR1+4 **v.** 3	spawners	W'90'91,S'90	.506	.704	.037	.511	.478	.348
e)TOR1+4+3**v.**2	sp, non	W'90,'91, S'90 Sp'90	.296	.173	.524	.987	.741	.874

Winter spawners collected in consecutive years from Torbay were not significantly different ($p>0.05$) except at *EST-4** ($p<0.05$). When these samples were pooled and tested for Hardy Weinberg equilibrium only *AAT** was significantly out of equilibrium ($p<0.01$) (Appendix 7.3.4). Given the uncertainty of the cause of the *EST-4** variation, a lack of differences at any other loci and conformity to Hardy Weinberg equilibrium, these samples were pooled as winter spawners for further comparisons.

Winter spawners and summer spawners were not significantly different ($p>0.05$) except the *EST-4** locus. When pooled they were in Hardy Weinberg equilibrium except *AAT** ($p<0.05$) (Appendix 7.3.4), so these samples were pooled as Torbay spawners.

The spawners and non-spawners collected from Torbay were not significantly different at any locus ($p>0.05$) but were kept separate for the spatial analysis.

SITE: Dunsborough (DUN), WA

Conformity to Hardy Weinberg equilibrium expectations of the Dunsborough samples is given in Appendix 7.1.5. All Dunsborough samples were in Hardy Weinberg

equilibrium (p>0.05), with the exceptions of DUN1, DUN2 and DUN4 at the *AAT** locus (p<0.05). Thus, each sample was considered to be homogeneous.

Table 6: Levels of significance of contingency chi-square tests between Dunsborough, WA samples. ? reproductive status unknown. See above for abbreviations.

COMPARISON	BREEDING STATUS	SEASON, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
a) DUN2 v. 3	spawners	S'90, S'90	.670	.622	.241	.436	.446	.172
d) DUN2+3v. 1	sp, ?	S'90, W'90	.752	.869	.267	.484	.411	.679
e) DUN2+3+1 v. 4	sp?,non-pre	S'90+W'90, S'91	.000	.660	.011	.890	.835	.617

Table 6 presents the temporal variation results for the Dunsborough samples following the hypotheses in section 3.2.1 however, one winter sample collected from Dunsborough could not be reproductively staged.

The samples of spawners collected in Summer, 1990 were not significantly different at any locus (p>0.05). Pooled summer spawners were not significantly different to the winter '90 sample (p>0.05). These samples were in Hardy Weinberg equilibrium at all loci (p>0.05) (except *AAT**) when pooled together (Appendix 7.3).5.

The pooled summer and winter sample was significantly different (p<0.05) to the summer non-spawners at the *AAT** and *EST-4** loci. A similar difference between the summer '91 sample from Fremantle also occurred at *AAT** locus (see next section) so DUN4 was not pooled.

SITE: Fremantle (FM), WA

Chi-square results for fit to Hardy Weinberg are given in Appendix 7.1.6 for the Fremantle samples. FM1 was significantly out of equilibrium (p<0.05) at *AAT**. *AH** and *EST-4** were also close at p=.065 and p=.061, respectively, so this sample may not be homogeneous. The other Fremantle samples were considered to be homogeneous (see Appendix 7.1.6).

There was only one sample of spawners collected from Fremantle, so non-spawners were compared first within winter seasons, and then between winter and summer before comparing to the spawners (Table 7).

Table 7: Levels of significance for contingency chi-square tests between Fremantle, WA samples. See above for abbreviations.

COMPARISON	BREEDING STATUS	SEASON, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
c) FM1 v. 3	non-sp	W'90, W'91	.359	.618	.840	.475	.090	.133
c) FM2 v. 3	non-sp	S'90, W'91	.288	.171	.004	.428	.220	.180
e) FM2+3 v. 4	non-sp, sp	W+S'90, S'91	.041	.229	.018	.438	.225	.685

There was no significant difference at any locus between non-spawning samples collected in consecutive winters. However, the homogeneity of FM1 was in doubt, FM1 and FM3 were significantly out of Hardy Weinberg equilibrium at *AAT**, *AH**, *EST-4** and *MPI** loci ($p<0.05$) (Appendix 7.3.6) and therefore not pooled.

The summer and winter non-spawners were not significantly different ($p<0.05$) except at the *EST-4** locus. As esterases are not reliable without other differences, these samples are not considered to be significantly different. When pooled, FM2+3 was in Hardy Weinberg equilibrium ($p>0.05$) except at the *AAT** and *MPI** loci ($p<0.05$) due heterozygote deficiencies (Appendix 7.3.6). As separate samples these loci were significant and pooling exacerbated the heterozygote deficit, so it is reasonable to pool these as a homogeneous sample.

The Fremantle non-spawners were significantly different to the Fremantle summer spawners at the *AAT** and *EST-4** loci ($p<0.05$). A similar difference was found within the Dunsborough samples which are geographically close so were left unpooled.

SITE: Cheynes Beach (CHB), WA

The Cheynes Beach sample, the only non-replicated sample from WA, was in Hardy Weinberg equilibrium except at the *AAT** and *AH** loci ($p<0.05$) due to heterozygote deficits (Appendix 7.1.7).

SITE: Boston Bay (BSA), S.A.

In the only sample from South Australia, *AAT** was out of Hardy Weinberg equilibrium ($p<0.05$) due to a heterozygote deficiency. *PEPB** was also out but due to a heterozygote excess ($p<0.05$) (Appendix 7.1.8) probably due to chance.

SITE: Port Phillip Bay and Lakes Entrance, Vic.

The first sample collected from Port Phillip Bay was in Hardy Weinberg equilibrium except for the *AAT** and *PEPB** loci ($p<0.05$). The second Port Phillip Bay sample was also in equilibrium except for *AAT** and *MPI** ($p<0.05$). Both the *PEPB** and *MPI**

differences were due to low expected values despite pooling (see Appendix 7.1.9). They are considered to be homogeneous samples.

The sample from Lakes Entrance was not in Hardy Weinberg equilibrium at the *AAT** and *PGM** loci ($p<0.05$). Both showed deficits with the expected numbers of heterozygotes. Despite the exclusion of specimens from the extreme ranges of this sample (section 2.2) there were still different size classes and therefore different year classes (see Appendix 4.0) so it may not be a homogeneous sample.

Table 8 gives the results for comparisons between years within Port Phillip Bay and then between those samples and the Lakes Entrance sample.

Table 8: Levels of significance for contingency chi-square comparisons between Victorian samples. See above for abbreviations.

COMPARISON	BREEDING STATUS	MONTH, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB*PGM**	
c)PPB1 v. 2	non-sp	F'91, Mar'92	.518	.891	.559	.161	.340	.383
c)PPB1+2 v. LKE1	non-sp	F'91, M'92, Aug'92	.560	.438	.865	.109	.940	.807

The Port Phillip Bay samples were not significantly different at any locus ($p>0.05$). These samples were in Hardy Weinberg equilibrium when pooled except for *AAT** and *PEPB** ($p<0.05$)(Appendix 7.3.7) however, *PEPB** was significant in PPB1 and the difference was exacerbated by pooling. These samples were pooled for subsequent analyses.

The Port Phillip Bay sample was not significantly different from the Lakes Entrance sample at any locus ($p>0.05$), but they were not pooled as the homogeneity of the latter sample was in doubt.

SITE: Jervis Bay (JB), NSW.

The JB1 sample was in Hardy Weinberg equilibrium at all loci ($p>0.05$) except the *AAT** locus (Appendix 7.1.10). This was regarded then, as a homogeneous sample. JB2 however, was significantly out of Hardy Weinberg equilibrium at four loci. This suggests that the sample may consist of two or more subpopulations (Appendix 7.1.10). JB2 was exceptional in that two previously unscreened alleles (only one occurrence of each) appeared at both the *AAT** and *PGM** loci.

Table 9: Levels of significance of contingency chi-square comparisons between Jervis Bay, NSW samples. See above for abbreviations. F=February.

COMPARISON	BREEDING STATUS	MONTH, YR	LOCI					
			*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
JB1 v. JB2	non-spawning	F '91, F'92	.743	.045	.785	.232	.182	.599

The samples from Jervis Bay were significantly different at the *AH** locus ($p<0.05$) (Table 9). These samples also differed in their sex ratios (see Table 2) but did not show a consistent pattern with the KGS and ESP samples with regard to frequencies and sex dominance.

SITE: South Africa (SAF)

The *AAT**, *MPI** and *PGM** loci were out of Hardy Weinberg equilibrium ($p<0.05$) due to heterozygote deficiencies (Appendix 7.1.11). *MPI** was attributable to a low expected value for the uncommon homozygote but with *PGM** out, it may be a mixture of subpopulations, or it may be due to the small sample size.

Table 10: Summary of homogeneous samples (that have been pooled) and their simplified code for the spatial analysis, the season they were collected in, spawning condition and sex ratio.
* see next section re pooling.

COMBINED SAMPLE	SPATIAL CODE	SEASON	SPAWNING CONDITION	SEX RATIO (F:M)
KGS5	KGS5	W '91	spawning	3.80:1
KGS6	KGS6	W '91	mixed spawn	0.88:1
TOR1+4+3	TOR	W'90+'90, S'90	spawn/nonspawn	3.70:1
CHB1	CHYB	W'91	spawn	1.26:1
BB1+3	BRBY	W '90+S '90	spawn/non-pre	3.33:1
DUN1+2+3		W'90+S'90,	? and spawn	1.29:1
FM4	DUNFREsp.*	S'91	spawn	2.00:1
DUN4		S'91	nonspawn	3.63:1
FM2+3	DUNFREnonsp.*	S'90, W'91	nonspawn	1.67:1
ESP3	ESP juv.	W '91	juveniles	
ESP2+6	ESP sum	S'90+'91	non-spawn	1.40:1
ESP4+5	ESP win	W '91	spawners	4.50:1
BSA1	S.AUST	W'92	non-prespawn	0.74:1
PPB1+2	PPB	Feb/Mar '91/'92	nonspawn	1.12:1
JB1	JB	Feb '91	nonspawn	1.69:1
SAF1	S.AFR	S'91	?	?
not included in spatial analysis but homogeneous samples				
KGS1		Au '90	non-spawning	0.61:1
TOR2		Sp '90	nonspawners	1.45:1
BB2		Sp '90	nonspawners	1.40:1

3.2.2 Spatial Variation between Sites

Samples collected from non-breeding periods in WA were excluded in the spatial analysis (see section 2.6). The pilchards collected from the other Australian states were not in spawning condition, but were included because no others were available; and it is unlikely that they would interbreed with fish from WA. Only samples that fitted Hardy Weinberg equilibrium expectations were included and it was assumed that those groupings are retained during spawning. Table 10 summarises the samples used and gives the revised codes of the pooled samples.

Between the Dunsborough and Fremantle samples significant temporal variation was found between spawners and non-spawners (Tables 6 and 7). Table 11 presents the results of comparisons between those samples. The Dunsborough and Fremantle spawners were not significantly different ($p>0.05$) at any locus and were in Hardy Weinberg equilibrium ($p>0.05$) (see Appendix 7.4.1). The non-spawners (D4 and FM23) were also not significantly different ($p>0.05$) (except at *EST-4**) but were not in Hardy Weinberg equilibrium (Appendix 7.4). The spawners and non-spawners were significantly different at the *AAT** locus ($p<0.001$) (and *EST-4**) and were therefore kept as separate samples. Both groups contained winter and summer samples and were predominantly female.

Table 11: Levels of significance for contingency-chi square comparisons between Dunsborough and Fremantle spawners and nonspawners.

COMPARISON	BREEDING STATUS	*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
D123 **v.** FM4	spawners	.755	.465	.180	.633	.594	.337
D4 **v.** FM23	non-spawners	.926	.720	.000	.495	.408	.677
D123F4 **v.** D4F23 (ie. DUNFREsp **v.** DUNFREnonsp)	sp v. non	.000	.631	.000	.824	.582	.681

As there were different subpopulations collected from the Albany region within a six week period, it was not possible to follow the strategy of Shaklee *et al.* (1990b) and systematically compare samples of closest geographic proximity to elucidate subpopulation boundaries. All WA spawning samples, the Esperance summer adult sample, and samples from South Australia, Victoria and New South Wales were compared in a UPGMA cluster analysis using Rogers (1972) genetic similarity coefficient. Those results for all loci are presented with goodness of fit statistics in Figure 4. The cluster analysis was also run without the *EST-4** data for comparison

(Appendix 9.0). The general grouping of samples was similar, the only difference was in the positioning of the Bremer Bay and South African samples.

Obvious groupings such as Dunsborough and Fremantle with Torbay and KGS5, Cheynes Beach with Bremer Bay and South Australia with Victoria are apparent (Figure 4). One anomaly was apparent in the grouping between KGS6 and JB1 which are geographically distant. The pairs of samples from those main groupings (except KGS6 and JB) were then compared. No significant differences were found between those pairs at any locus ($p>0.05$) (Table 12A).

The Dunsborough, Fremantle and Torbay (DFT) group conformed to Hardy Weinberg equilibrium expectations when pooled (see Appendix 7.4.3). The Bremer Bay (BB) and Cheynes Beach (CHB) samples were not homogeneous at all loci. The alternative grouping of Cheynes Beach and Esperance (Appendix 9.0) were close to significance at the *PGM** locus, so BB and CHB were kept together for subsequent comparisons. The South Australian and Victorian samples were significantly out of Hardy Weinberg so were not pooled for subsequent comparisons (Appendix 7.4.3).

TABLE 12**A**: Levels of significance of contingency chi-square comparisons and subpopulation groupings between: i) closest cluster analysis groupings; and then between ii) Dunsborough, Fremantle and Torbay (western group) and other WA south coast samples.
12 **B**: Levels of significance of contingency chi-square comparisons between:
i) South Australia and other WA south coast sites: and
ii) Victoria and New South Wales.

COMPARISON	new code	*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**	subpopulation
A								
DUNFRE **v.** TOR	(DFT)	.270	.554	.236	.265	.492	.489	western
CHB1 **v.** BB13	(CHBB)	.185	.171	.092	.895	.788	.840	s-western
S.AUST **v.** PPB		.589	.955	.059	.847	.817	.708	s-eastern
DFT **v.** CHBB		.058	.202	.000	.607	.781	.023	
DFT **v.** KGS5		.287	.499	.009	.503	.940	.524	western
DFT **v.** KGS6		.001	.013	.703	.739	.836	.056	
CHBB **v.** KGS5		.716	.095	.971	.926	.684	.013	
CHBB **v.** KGS6		.120	.230	.005	.791	.474	.697	s-western
B								
S.Aust **v.** CHBB		.391	.315	.000	.991	.665	.092	
S.Aust **v.** ESPwin		.746	.705	.027	.357	.234	.058	
S.Aust **v.** ESPsum		.188	.122	.021	.859	.824	.907	sth-central
JB **v.** PPB		.026	.025	.337	.251	.481	.866	eastern
and JB1+2 **v.** PP12		.079	.122	.696	.289	.439	.786	sth-eastern

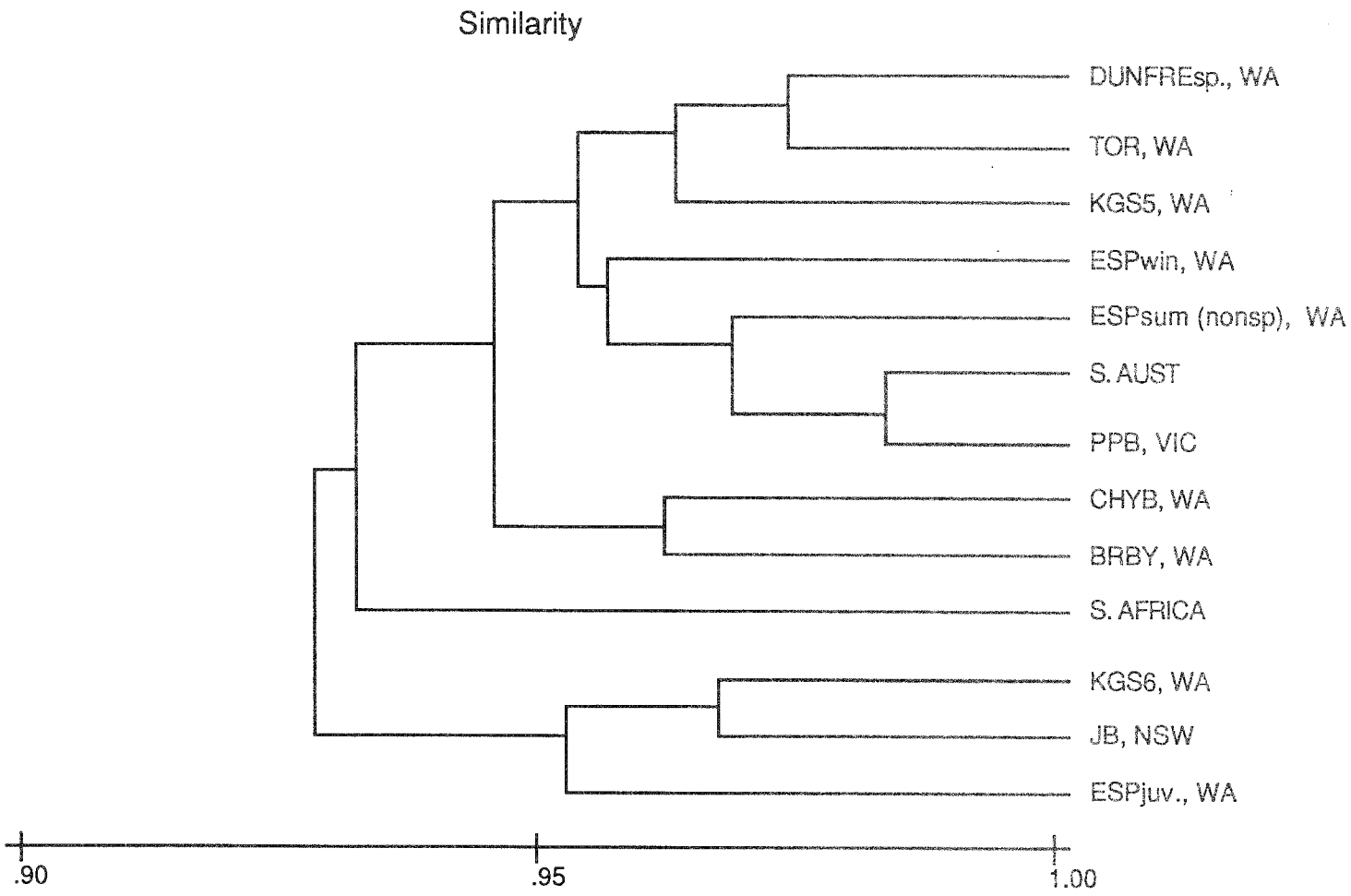


Figure 4: Dendrogram of genetic interrelationships of the Australian pilchard (*S. s. neopilchardus*) and a sample of *S. ocellata* from South Africa, based on the analysis of 6 polymorphic loci using Rogers '(1972) genetic similarity coefficient, as calculated using the UPGMA method, by BIOSYS-1. Site and sample codes are given in Table 10.
Goodness of fit statistics: Farris (1972) "f" =.923; Cophenetic correlation =.652; Prager and Wilson (1976) "F" = 1.258;
Percent standard deviation (Fitch and Margoliash, 1967) = 1.789

The second part of Table 12A shows that there are two main subpopulations in WA which overlap in the vicinity of Albany. The combined Dunsborough, Fremantle and Torbay samples were not significantly different to KGS5 so they were grouped together as the "western" subpopulation. However, the two groups were significantly different at *EST-4**, so maybe they breed together but do not feed together (see section 4.1). The KGS6 sample was significantly different to the DFT sample and KGS5 (see Table 1), but was not significantly different to the combined Cheynes Beach and Bremer Bay so they were grouped together as the south-western subpopulation.

The Esperance juveniles were not grouped with any particular samples (Figure 4) but were closest to the KGS6 sample. The Esperance juveniles were significantly different to the western subpopulation ($p<0.05$), to the mid south-western subpopulation, and not significantly different to the South Australian sample ($p>0.05$) (but *PGM** was close) (see Appendix 10.0). The most likely source of the Esperance juveniles seems to be the Esperance summer non-spawners (see Table 2).

The South Australian sample from Boston Bay, was grouped closely with Victorian samples but, as it was not significantly different to the CHBB group nor, the Esperance adult samples ($p>0.05$) (although it was almost significant to the Esperance winter sample at the *PGM** locus, Table 12b), it may also be considered as part of the south-western group.

Jervis Bay and Port Phillip Bay were significantly different at *AAT** and *AH** ($p<0.05$) (Table 12b) suggesting subdivision between the east coast, that is, there is an eastern and south-eastern subpopulation. However, when JB2 was included there were no significant differences, and the Lakes Entrance sample was heterogeneous thus, mixing occurs between these subpopulations on the south east coast of Australia.

There were no fixed differences between the South African pilchards and any of the Australian pilchards (Figure 4). However, there were differences in allele frequencies between the two groups. South Africa was more clearly separated when the *EST-4** data was excluded (Appendix 9.0).

4.0 DISCUSSION

This study supports the findings of Blackburn (1951) and Syahailatua (1992) that Australian pilchards do not comprise one large interbreeding population. On a local scale there was significant temporal variation, such as that found within King George Sound (KGS). The following discussion first addresses the problems with the *EST-4** and *AAT** loci and then, the temporal and spatial variation results.

4.1 Locus Specific Variation

There was significant temporal variation at the *EST-4** locus within WA sites but not between samples from the eastern states. Esterase variation was not considered to be sufficiently reliable to discern subpopulations without other variable loci, in view of the following. It may indicate for example, feeding groups rather than breeding groups.

Berrebi *et al.* (1990) state that "fish esterases are among the most difficult enzymes to identify using starch gel electrophoresis because of the many loci that are simultaneously active, the duplication phenomena, satellite bands and stain traits". Esterases act on externally derived substrates (Smith 1979) and the frequencies have been shown to fluctuate through time (Seeb *et al.* 1990), vary according to diet (Guerin and Kerambrun 1982 and Kerambrun and Guerin 1983), be affected by thermal shock (Kerambrun and Guerin 1981), vary between marine and estuarine populations in relation to salinity fluctuations and food availability (Sin and Jones 1983) and are reportedly influenced by environmental and ontogenetic factors (Utter *et al.* 1974, Allendorf and Utter 1979 both cited in Andersson *et al.* 1981).

Smith (1979) found that year classes of the New Zealand snapper, *Chryophrys auratus*, spawned in warm years differ in esterase frequencies to those spawned in cold years. He proposed that there is differential mortality of larval genotypes between warm and cold years. The selective agent may not be temperature but an associated physical parameter or a biological variable such as the food type available (Smith 1979).

The TOR1 and TOR4 samples of this study for example, were collected in the same area and season but in different years (Appendix 1). Those samples were significantly different at the *EST-4** locus. That may indicate that those samples were from different schools that fed in different areas, and/or were born in different years. WA fishermen previously used pollard to attract pilchards to the surface, particularly around Bremer Bay, and to a lesser extent, Albany (Fletcher pers. comm.). The specimens from many of those samples had severely distended guts which had disrupted other internal organs. We did not note the presence of pollard in the earliest samples and thus, could not check if its

presence was related to the *EST-4** frequencies. Future work could include an investigation of the relationship between gut contents and esterase allele frequencies.

Most samples had a large heterozygote deficit at the *AAT** locus. Grant (1985) also examined a polymorphic *AAT** locus in the southern African pilchard, *S. ocellata*. We could not determine if it was the same locus and Grant made no specific reference about his *AAT** locus and degree of fit to Hardy Weinberg equilibrium. Richardson *et al.* (1986) have outlined a number of reasons for locus specific phenomena, such as misscoring, strong selection, the presence of null alleles and assortive mating (more than one process may operate simultaneously).

Many samples varied in the ratio of females to males but the loci screened were not sex linked and temporal variation within KGS was not related to the predominance of sex in a sample. Thus initially, we considered misscoring to be the most likely cause for that heterozygote deficit due to the difficulty experienced scoring this locus as a result of rapid overstaining. However, this stain was usually scored twice (see section 3.1) and each was rechecked with negatives taken of the zymogram before overstaining occurred.

The temporal variation results also suggest that the misscoring may not have been the most likely cause. For example, the Esperance juvenile sample which was likely to be the most unmixed sample in the study, conformed to Hardy Weinberg equilibrium expectations at the *AAT** locus. Furthermore, significant differences between *AAT** loci only occurred between the lower west coast spawners and non-spawners; and between the KGS male dominated spawners and non-spawners.

Smith (1979) explained that a large sample containing a number of year classes could show an excess of homozygotes, mimicking population mixing if there have been different selective values between successive year classes. He noted that this would be particularly evident in short-lived species, where samples would be made up of only a few dominant year classes. Pilchard samples in this study were comprised of dominant year classes which could not be separated due to sample size/statistical constraints (see Appendix 4.0). Thus, any future genetic work on *S. s. neopilchardus* should investigate *AAT** variation to determine the process responsible for the large heterozygote deficits before making any firm conclusions based on differences at that locus.

4.2 Temporal Variation within Sites

Many samples did not conform to Hardy Weinberg equilibrium. According to Richardson *et al.* (1986) varying levels of heterogeneity at different loci and in different samples, as we found, suggest that widely distributed subpopulations were involved. Most WA sites exhibited temporal variation between homogeneous samples. That variation, and the

occurrence of heterogeneous samples, indicated that pilchard subpopulations mix during their life cycle (that is, a Wahlund effect).

Kornfield *et al.* (1982) also found intra-locality variation within Atlantic herring populations. Much of the temporal variation between their fall spawners occurred at the *AH** and *PGM** loci as we found in the Australian pilchard. Grant (1985) detected two polymorphic *PGM** loci be we detected only one, Grant's *PGM-2** locus appeared to be similar to our *PGM** locus but he had six allelic variants (we had four and one instance of a fifth allele in the JB2 sample). Our sample of *S. ocellata* was comparable to *S. s. neopilchardus*. Kornfield *et al.* (1981) have demonstrated the simple Mendelian inheritance of the *PGM** locus (and others) via breeding experiments.

The difference between the KGS winter '91 samples and the intermediacy of the KGS April '90 sample indicated that there are different subpopulations that frequent King George Sound. The heterogeneous samples indicate that mixing occurs in the Albany region. Fletcher *et al.* (1992) have surmised that increased winter catches at Albany are due to an intense aggregation of a relatively small stock off that region, a theory also supported by historical data. However, it may be an aggregation of subpopulations which do not spawn together. There is a lack of information about the actual extent and duration of mixing during the life cycle that occurs between adult pilchards. Tagging data would provide useful information to resolve those questions.

The Esperance winter spawners were different to the summer non-spawners and the juveniles but the latter two samples were not different. It seems then, that Esperance is inhabited by at least two different subpopulations, in different seasons. However, it was not apparent when and where the summer adults spawned. There were also signs of subpopulation mixing in the Esperance region.

There was no evidence to suggest that the summer and winter spawners collected from Bremer Bay were different subpopulations, but there was variation between the spawners and non-spawners collected in different seasons around Bremer Bay. It appears that Bremer Bay is a site where different subpopulation(s) feed during non-breeding seasons.

Torbay appeared to be a relatively stable site as there was no temporal variation found between any samples from there.

There was significant variation between combined Dunsborough and Fremantle spawners and non-spawners but that variation was only at the *AAT** locus which may not be reliable without other variable loci.

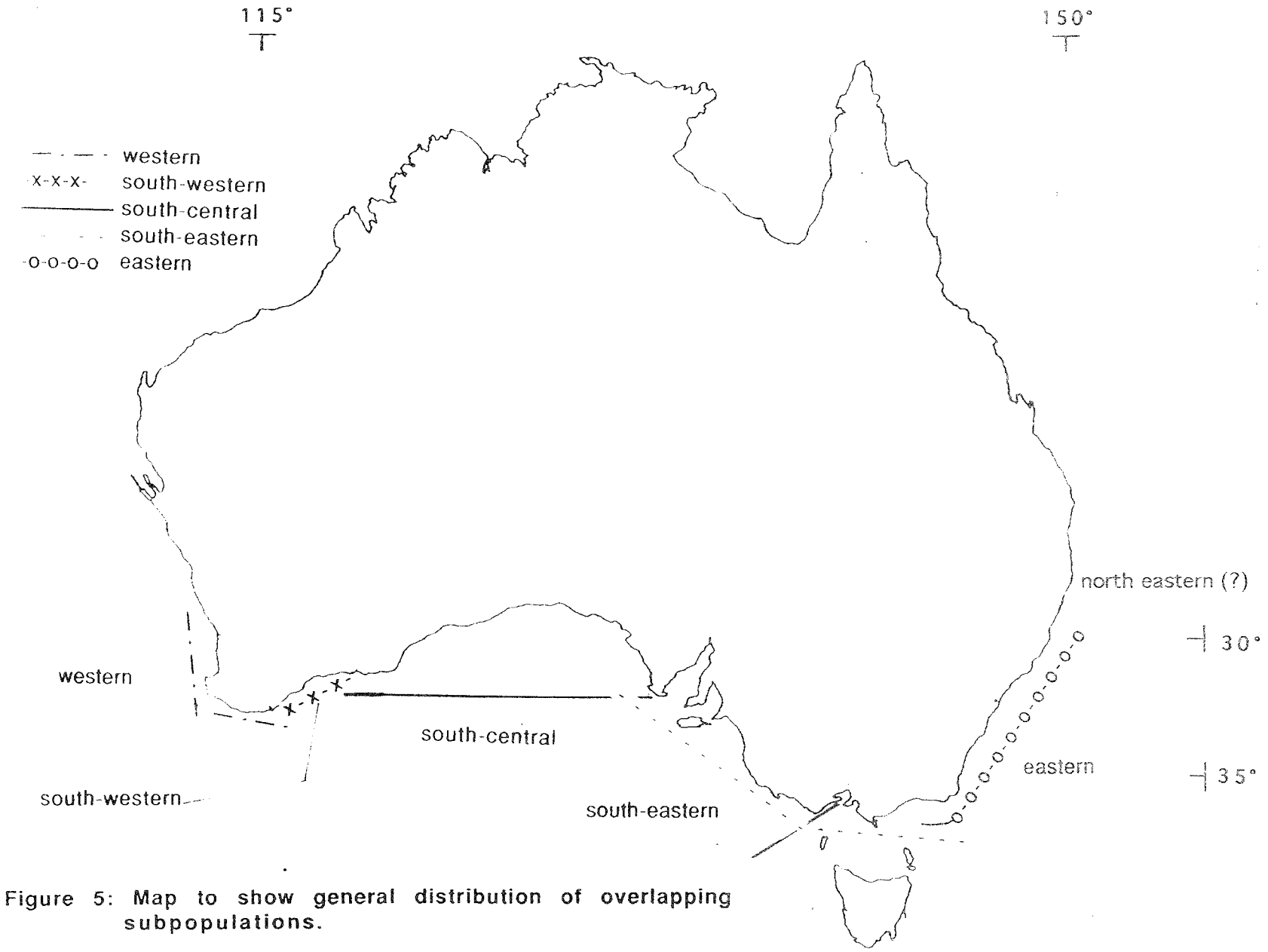


Figure 5: Map to show general distribution of overlapping subpopulations.

On the east coast of Australia, no temporal variation was found among the Victorian samples but, there was between the Jervis Bay samples. However, we did not sample intensively in either of these areas.

The temporal variation between KGS winter spawners and the heterogeneity of the summer spawners precluded comparisons between spawners of those seasons. However, no evidence of discrete winter and summer spawning subpopulations within Bremer Bay and Torbay was found on the mid-south coast of WA. Kornfield *et al.* (1982) found significant variation between winter and summer spawners in the Atlantic herring at similar loci to this study. However, Ryman *et al.* (1984) recalculated those data and claimed that only .2% of the total gene diversity was explained by differences between spring and fall spawners. Furthermore, it has been found that herring may shift the time of spawning in response to environmental changes (Anokhina 1971, cited in Ryman *et al.* 1984).

Parrish *et al.* (1989) claim that sardines make extensive migrations and have extremely large populations and thus, concluded that we do not see sympatric or parapatric species of sardines which can be interpreted to have arisen *in situ*. This is not the case with the Australian pilchard where an individual's range is considered to be hundreds not thousands of miles (Blackburn 1951 and Fletcher and Tregonning in press, cited in Fletcher *et al.* 1992) the pilchard stock off Albany for example, is not large (Fletcher 1992) and we have found evidence of sympatry within KGS.

4.3 Spatial Variation

The spatial variation results indicated that there are contiguous subpopulations around the Australian coast which sometimes overlap and can be grouped into five general geographic "stocks" (see Figure 5). Blackburn (1951) discerned three groups of pilchards in Australian waters (see section 1). The delineation of those eastern, south-eastern and south-western "stocks" was based on growth rates (high, low and intermediate respectively), number of vertebrae and abundance. Blackburn claimed that the boundary zone between the eastern stocks is near the New South Wales-Victoria border and is maintained by different breeding times. This study supports that claim as the second Jervis Bay (JB2) sample appeared to be under the Wahlund effect (ie. a mixture of two or more subpopulations). Furthermore, there was significant variation between the homogeneous Jervis Bay and Port Phillip Bay (PPB) samples, but not when the mixed JB2 sample was included. The Port Phillip Bay and Lakes Entrance samples were not significantly different, but the latter was heterogenous encompassing several size classes. As we know that there is genetic and morphologic variation between the JB

and Victorian samples, we also refer to them as the eastern and south-eastern stocks, respectively (see Figure 5).

Blackburn (1951) also contended that there were at least two groups within the eastern stock which meet between Port Jackson and Jervis Bay. Syahailatua's (1992) study also suggested two eastern morphologic groups. The second Jervis Bay sample was heterogeneous also suggesting the existence of two or more subpopulations, but we could no confirm that due to the unreliability of the Wooli sample. Any future study should examine samples from Wooli and Eden to clarify the degree of genetic subdivision on the east coast.

Blackburn (1951) was uncertain whether his South Australian (SA) samples belonged to the south-eastern stock and/or his south-western stock. The allozyme results indicated that the SA sample in this study was not significantly different to either adjacent samples (viz: PPB and Esperance) but, it was almost significantly different to the Esperance winter spawners and it was heterogeneous when pooled with the PPB samples. However, Figure 4 indicated that it is closer to the south-eastern than the south-western samples. As it is not a genetically distinct subpopulation and the Flinders current runs west between Victoria and South Australia (Syahailatua 1992) which may allow some south-eastern individuals to spawn in SA but it is geographically distant, we refer to it as the south-central "stock".

The subpopulations discerned in this study also generally conformed with the six morphologically distinct groups described by Syahailatua (1992) and presented in Figure 6. Because Rogers' (1972) distance (and similarity) is equivalent in principle to Mahalanobis' distance for morphological characters (which Syahailatua used), the relative distance between population units calculated from the morphometric and allelic data are comparable (Richardson *et al.* 1986) (but in this case, there are scale differences on the axes).

Syahailatua (1992) showed that pilchards of the west and south coasts of WA were morphologically different; and all the WA south coast samples belonged to one morphologic group. However, we found overlap between samples from the west (Dunsborough and Fremantle), and south-west coasts (Torbay and KGS5), (ie. the western subpopulation).

In the discriminant analysis of the morphometric and meristic variables, the most misclassified individuals were between the lower-west coast and the south coast of WA (Syahailatua, 1992 pg: 24). That percentage of individuals moving between the lower west coast and the south west coast (around to the Walpole area, say) would be enough in

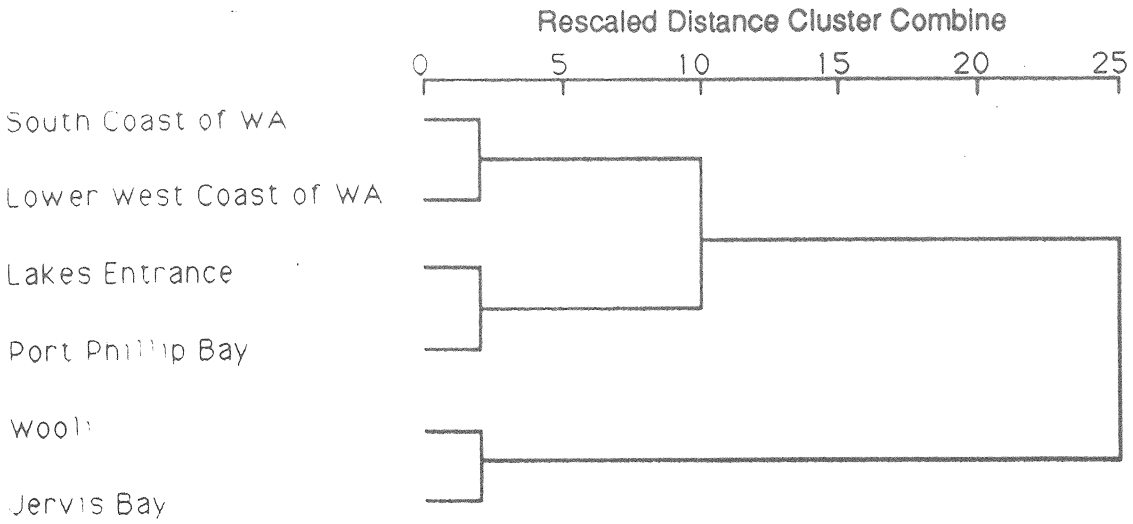


Figure 6: Dendrogram of morphometric inter-relationships of the Australian pilchard. based on a cluster analysis of the group centroids on the five possible discriminant functions using Mahalanobis distance.
(Source: Syahailatua 1992).

genetic terms to render them genetically indistinct. We also discerned a genetically distinct group on the mid south-west coast (the south-western subpopulation) around Bremer Bay and Cheynes Beach. Evidence of mixing of both subpopulations within the Albany region was found. Morphometric and meristic data typically exhibit broad overlap between stocks and are sensitive to environmental conditions (Smouse *et al.* 1990 and references therein). That is, the morphometric study discerned two distinct morphological groups within WA waters, and the allozyme study which was based on a more intensive sampling program, has shown that those morphologic groups may also be genetically distinct subpopulations but are not confined to specific coasts, or even specific sites through time.

In contrast to this study, Ryman *et al.* (1984) reported many morphologically distinct stocks of eastern Atlantic herring but little genetic variation. That resource has been exploited for hundreds of years (Suthers pers. comm.). The western Atlantic herring have more genetic variation and have been exploited since 1961 before a crash in 1977 (Stephenson and Kornfield 1990). Hedgecock *et al.* (1989) did not find signficiant variation between Gulf of California and Pacific sardines (*S. sagax caeurlea*) and concluded that it was unlikely that the collapse of that fishery caused a severe enough bottleneck to have resulted in reduced variation. However, variation may not have been detected because that study was based on very small sample sizes, no assessment was made of reproductive status of specimens, and samples were not replicated. Furthermore, samples were not collected simultaneously so there is no guarentee that they had not sampled from the same school of fish. The Australian pilchard has been exploited more recently, and there has been no reported population crashes. Thus, heavy exploitation, to the extent of a population crash, may result in a loss of genetic diversity. It would therefore be of considerable value to examine the correlation between the degree of exploitation and the amount of genetic variation in the Clupeidae.

The subpopulations east of WA appear to be kept independent by different spawning seasons (see section 1). Blackburn (1951) thought that pilchards on the east coast could spawn twice but found no evidence of them doing so. However, there appears to be a more complex mechanism maintaining the population structure on the south coast of WA where there are two breeding seasons. The next section suggests a possible mechanism.

Nutrient levels in WA coastal waters are largely dependent on terrestrial inputs because upwelling is not a feature of the Leeuwin Current (Lenanton *et al.* 1991). Most WA rainfall occurs in winter so there is likely to be greater food availability in that season. The Leeuwin Current peaks in winter and is weak in summer (Morgan and Wells 1991). Catches are traditionally poor in WA during the summer season (Fletcher *et al.* 1992). Unlike the other WA south coast sites, only winter spawners were collected from

Esperance, so it is possible that spawning incidence is dependent on salinity and temperature cues and/or food availability associated with the intensity of the Leeuwin Current.

KGS and Bremer Bay (and to a lesser extent Walpole) are areas where adult densities are high and concentrations of eggs have been found in the month of July which is the main WA south coast spawning period (Fletcher *et al.* 1992). Our samples were collected about one month prior to that (and were probably still "pre-spawners") so, it is possible that pilchards aggregate in KGS before some migrate to a different spawning area. For example, the KGS April spawners were grouped with the western subpopulation and there is only one known spawning site between the west coast and KGS, at Walpole (see Figure 2) (Fletcher *et al.* 1992). It is possible then, that the April "spawners" (KGS5) were en route to spawn at Walpole.

The KGS6 winter spawners were grouped with the south-western subpopulation so some Albany pilchards are not isolated from Bremer Bay. That subpopulation could be the resident subpopulation of KGS. Fletcher *et al.* (1992) contend that there is no chance that pilchards between Albany and Bremer Bay are reproductively isolated (but the adults may still act independently) because a lack of larvae in the Albany region in July of 1989 and 1990 was attributed to an easterly movement of larvae along the shelf. However, if the Albany eggs are moving across as a cohesive group, then it is possible that the Bremer Bay larvae have also moved away from the initial spawning site and been replaced by larvae from Albany.

The eggs and larvae to the east of KGS (toward BB) may be outside the influence of the Leeuwin Current because it flows at about 3 knots on the shelf and at about one knot up in the Bremer Bay area (Fletcher *et al.* 1992). Thus those larvae may be retained within that region outside the Leeuwin Current influence and develop as inshore juveniles whereas, the eggs spawned in the Walpole area may become entrained in the Leeuwin Current and be transported across towards Esperance.

The Esperance adult samples which were almost significantly different (Table 3) were both grouped between the western and south-western subpopulations in the cluster analysis. The Esperance summer adults were not different the South Australian sample were grouped in geographic terms as the south-central "stock". The winter adults however, appear to be part of the south-western subpopulation. The distribution of eggs and larvae and therefore the amount of mixing, between Bremer Bay and Esperance is unknown (Fletcher *et al.* 1992).

How then, can pilchards collected from the same site be reproductively isolated? The west Atlantic herring have such predictable spawning areas that homing by the spawning population has been inferred (Stephenson and Kornfield 1990, Iles and Sinclair 1982 and references therein). Fletcher *et al.* (1992) noted that the areas where major estuaries flow into the ocean on the south WA coast viz: Walpole, Albany and Bremer Bay (see Figure 2) are the sites of winter spawning centres. Instances of sardine and anchovy spawning activities concentrated in European waters enriched by estuarine outflow have also been noted (Fletcher *et al.* 1982 and references therein).

The fidelity to particular spawning areas by *S. s. neopilchardus* is unknown but the temporal and spatial results do not dispute that homing to estuaries could occur. Homing to enriched waters in low productivity areas, such as south western Australia, would help ensure that eggs and larvae can develop in areas with an adequate food supply. It has also been postulated that the sardines off the Pacific Northwest and southern California were distinct, either genetically or due to a strong tendency for fish to favour areas in which they were born (Radovich 1962, 1981 cited in Parrish *et al.* 1989). However, there are conflicting opinions regarding the existence of different subpopulations in the Californian Current (Parrish *et al.* 1989 and references therein). If homing does occur in *S. s. neopilchardus*, and females are more successful than males, that may explain the excess of females in some homogeneous samples. The homing hypothesis and environmental suitability for biannual spawning warrants further investigation.

Data suggesting that tidally or other geographically stable retention mechanisms are associated with the spawning area of each particular Atlantic herring stock and that the number of such retention areas determines the number of genetically distinct herring stocks were presented by Iles and Sinclair (1982). It is not known whether such mechanisms can, or do, exist on the south coast of WA. However, the homogeneous sample of Esperance juveniles (approx. one year old, Fletcher pers. comm.) indicate that larvae retain cohesive groups for at least a year. Additional samples of juveniles collected simultaneously from different sites of WA would be useful to clarify: i) if different subpopulations of juveniles exist in association with particular estuaries and ii) the cohesiveness of any such subpopulations.

4.4 *Sardinops ocellata* v. *S. s. neopilchardus*

Parrish *et al.* (1989) summarised the stock structure of sardines (pilchards) from the major current systems. For example, in the Benguela Current, significant allozyme variation was not detected between two supposed stocks which had been generally accepted on the basis of tagging, spawning location and landing data. However, the samples in the allozyme study (Grant 1985) were not replicated from the same sites; there were

significant deviations from Hardy Weinberg equilibrium, but no data for those tests were presented; and samples were not collected at the same time, so it is possible that geographic comparisons were made between mixed samples such that subpopulations could not be discerned.

Parrish *et al.* (1989) concluded that *Sardinops* is a monotypic genus and proposed that *Sardinops ocellata* and *S. s. neopilchardus*, among others, become synonyms of *Sardinops sagax* (Jenyns). The lack of fixed differences between the south African *S. ocellata* sample and *S. s. neopilchardus* suggests that they are not a different species but separate subpopulations, despite obvious morphological differences. However, a lack of electrophoretic differences does not necessarily mean that two groups are the same species. Fixed differences at approximately 20% of loci is indicative of different species (Richardson *et al.* 1986).

Our esterase zymogram for *S. ocellata* was different to the one described by Thompson and Mostert (1974) but not different to our *S. s. neopilchardus EST-4** zymograms. Thompson and Mostert (1974) and Le Clus (1978) found an excess of esterase homozygotes in *S. ocellata* but we found a perfect fit to Hardy Weinberg expectations. Thompson and Mostert (1974) and Le Clus (1974) found significant differences in esterase frequencies of the southern African pilchard, *S. ocellata*, among three sites, but Grant (1985) suggested that as there were large departures from Hardy Weinberg equilibrium their esterase variation may not have had a simple genetic basis.

When the cluster analysis was run without the *EST-4** data, the South African sample was better separated. If the *EST-4** frequencies were directly influenced by environmental conditions, *a priori* we could expect the South African pilchards to be further separated with the inclusion of the of the *EST-4** data due to inter-continental environmental variability.

5.0 CONCLUSION and MANAGEMENT IMPLICATIONS

This study has indicated that a series of contiguous quasi-independent pilchard subpopulations, (quasi, because mixing occurs at some stages) inhabit Australian waters. They are temporally unstable and it is unclear how much mixing occurs during their life cycle. The intensity of the Leeuwin Current in a given year may also influence where WA subpopulations are found in particular years.

Blackburn (1951) explained that years of relative abundance or scarcity will not necessarily be the same in all stocks, or even in adjacent stocks. Furthermore, small local stocks may be depleted by large-scale mobile fishing efforts (Iles and Sinclair

1982) and so, it is important to take the subpopulation structure into management considerations.

It is difficult to effectively regulate individual subpopulations (now referred to in a management context, as stocks) in a mixed-stock fishery, particularly when the degree of mixing is unknown at all life history stages. Such difficulties have been experienced in the North Sea for example, and consequently, biological stock questions have tended to be neglected and viewed as being largely irrelevant to the nature of the practical management unit (Iles and Sinclair 1982). It is often difficult to restrict catches from endangered or depleted stocks without limiting the entire fishery (Waples *et al.* 1990).

Iles and Sinclair (1982) suggest that in an mixed-stock complex, total effort must be restricted to a level much lower than would be appropriate for the individually assessed stocks or alternatively, severely limit the fishery to the non mixed spawning areas. The second option is not viable for the Australian pilchard because spawning areas also have mixed stocks at different times of the year.

The Genetic Stock Identification (GSI) procedure based on maximum likelihood estimation (MLE) has been used in the analysis of mixed stock fisheries such as Pacific salmon (Waples 1990, Waples *et al.* 1990 and Shaklee *et al.* 1990c and references therein). With adequate mixture and baseline samples, composition estimates for each stock can provide reliable information about the relative contribution of different stocks (Waples *et al.* 1990 and Shaklee *et al.* 1990c). However, estimates cannot be made for individual stocks that are too similar. GSI analyses have provided a more precise and comprehensive overview of stock structuring than by conventional tagging techniques (Waples *et al.* 1990). However, this has only been applied to fisheries where there is considerable archives of electrophoretic data which currently do not exist for the Australian pilchard.

6.0 RECOMMENDATIONS for FURTHER RESEARCH

i) Collect samples in July, the winter spawning season and again in summer spawning months, from the WA spawning sites of Walpole, KGS, Bremer Bay and Esperance.

ii) Sample intensively along the east coast of Australia to determine the number of subpopulations and how much overlap occurs between them

iii) Replicate sampling from South Australia and from sites between Esperance and Port Lincoln, South Australia.

iv) Collect more juvenile samples from sites where estuaries run into the sea (ie. potential spawning sites) to examine the cohesiveness of schools.

v) Conduct a tagging study to determine the amount of mixing and migratory movements.

REFERENCES

ANDERSSON, L., RYMAN, N., ROSENBERG, R. and G. STAHL (1981). Genetic Variability in Atlantic Herring (*Clupea harengus harengus*): Description of Protein Loci and Population Data. Hereditas **95**: 69-78.

BAKER, A.N. (1972). Reproduction, Early Life History and Age-Growth Relationships of the New Zealand Pilchard, *Sardinops neopilchardus,* (Steindachner). Fisheries Res. Div. N.Z., Fish. Res. Bull. **5**, 64p.

BERREBI, P., LANDAUD, P., BORSA, P. and J.F. RENNO (1990). Esterases of the Flounder (*Platichthys flesus*, Pleuronectidae, Teleostei): Development of an Identification Protocol using Starch Gel Electrophoresis and Characterization of Loci. Experentia **46** (8): 863-867.

BLACKBURN, M. (1949) The Age, Rate of Growth, and General Life History of the Australian Pilchard (*Sardinops neopilchardus*) in NSW waters. C.S.I.R.O. Aust. Bull. No: 242.

BLACKBURN, M. (1950). Studies on the Age, Growth and Life History of the Australian Pilchard, *Sardinops neopilchardus* (Steindachner), in Southern and Western Australia. Aust. J. Mar. Freshw. Res. **1**: 221-258.

BLACKBURN, M. (1951). Races and Populations of the Australian Pilchard, *Sardinops neopilchardus* (Steindachner). Aust. J. Freswat. Res., **2**(2): 179-192.

CHAPMAN, R.W. and B.L. BROWN. (1991). Mitochondrial DNA Isolation Methods. In: Whitmore D.H. (ed.) Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management. CRC Press. Boston.

DREDGE, M.C.L. (1969). Aspects of the Biology of the Australian Pilchard, *Sardinops neopilchardus* (Steindachner) Relating to Commercial Exploitation of Stocks in South Australia. Unpublished Honours Thesis, Univ. of Adelaide.

FARRIS. J.S. (1972). Estimating Phylogenetic Trees from Distance Matrices. Am. Nat. **106**: 645-668.

FITCH, W.M. and E. MARGOLIASH (1967). Construction of Phylogenetic Trees. Science. **155**: 279-284.

FLETCHER, W.J. (1990). A Synopsis of the Biology and the Exploitation of the Australasian Pilchard, *Sardinops neopilchardus* (Steindachner) Part 1: Biology. Fisheries Research Report #88.

FLETCHER, W.J. (1991). A Synopsis of the Biology and the Exploitation of the Australasian Pilchard, *Sardinops neopilchardus* (Steindachner) Part II: History of Stock Assessment and Exploitation. Fisheries Research Report #91.

FLETCHER, W.J. (1992). Use of a Spatial Model to Provide Initial Estimates of Stock Size for a Purse Seine Fishery on Pilchards (*Sardinops sagax neopilchardus*) in Western Australia. Fisheries Research **14**: 41-57.

FLETCHER, W.J., TREGONNING, R.J., SANT, G.J., BLIGHT, S.J. and M.H. ROSSBACH. (1992). Investigation of the Abundance and Distribution of Pilchard Eggs and Larvae of South Western Australia. FIRDC No. 91/24.

FLETCHER, W.J. and R.J. TREGONNING (1993). The Distribution and Timing of Spawning by the Australian Pilchard (*Sardinops sagax neopilchardus*) off Albany, Western Australia. Aust. J. Mar. Freshw. Res. In press.

GRANT, W.S. (1985). Population Genetics of the Southern African Pilchard, *Sardinops ocellata*, in the Benguela Upwelling System. Int. Symp. Upw. W. Afr., Inst. Inv. Pesq., Barcelona. **1**: 551-562.

GUERIN, J.P. and P. KERAMBRUN (1982). Effects of Diets on Esterases, Alkaline Phosphatase, Malate dehydrogenase and Phosphoglucomutase Activity Observed by Polyacrylamide Gel Electrophoresis in *Tisbe holothuriae* (Harpacticoid copepod). Comp. Biochem. Physiol. B. **73B**(4): 761-770.

HARRIS, H. and D.A. HOPKINSON (1976). "Handbook of Enzyme Electrophoresis in Human Genetics." North Holland, Amsterdam.

HEDGECOCK, D., HUTCHINSON, E.S., LI, G., SLY, F and K. Nelson. (1989). Genetic and Morphometric Variation in the Pacific Sardine, *Sardinops sagax caerulea*: Comparisons and Contrasts with Historical Data and with Variability in the Northern Anchovy, *Engraulis mordax*. Fishery Bulletin, **87**(3): 653-671.

ILES, T.D. and M. SINCLAIR (1982). Atlantic Herring: Stock Discreteness and Abundance. Science **215**: 627-633.

JOSEPH, B.D.L. (1981). Pilchard Fishery at Jervis Bay - Biology, Fishery and Population Dynamics. Unpublished MSc. Thesis. U.N.S.W.

KERAMBRUN, P., and J-P. GUERIN (1981). Influence of Thermal Reduction Kinetics After Thermal Shock on Modifications in the Activities of Esterases and the Possibilities of Survival of *Scolelepis* (Malacoceros) *fuliginosa* (Polychaete Annelid). Mar. Environ. Res. **5(2)**: 145-156.

KERAMBRUN, P., and J-P. GUERIN (1983). Esterase and Malate Dehydrogenase Zymogram Modifications as a Function of Diet in the Harpacticoid Copepod, *Tisbe holothuriae*. Rapp. P. V. Reun. Ciesm. **28**(6): 173-175.

KORNFIELD, I., GAGNON, P.S., and B.D. SIDELL. (1981). Inheritance of Allozymes in Atlantic Herring (*Clupea harengus harengus*). Can. J. Genet. Cytol. **23**: 715-720.

KORNFIELD, I., SIDELL, B.D., and P. S. GAGNON. (1982). Stock identification in Atlantic herring (*Clupea harengus harengus*): Genetic Evidence for Discrete Fall and Spring Spawning Populations. Can. J. Fish. Aquat. Sci. **39**: 1610-1621.

Le CLUS, F. (1978). Allele Combinations Responsible for Heterozygote Deficiency in Esterase Polymorphism of the Pilchard, *Sardinops ocellata*. Fishery Bull. Un. S. Afr. **10**: 1-9.

LENANTON, R. C., JOLL, L., PENN, J. and K. JONES. (1991). The Influence of the Leeuwin Current on Coastal Fisheries of Western Australia. Journal of the Royal Soc. of W.A. **74**: 101-114.

MORGAN, G.J. and F.E. WELLS (1991). Zoogeographic Provinces of the Humboldt, Benguela and Leeuwin Current Systems. J. Royal. Soc. W.A. **74**: 59-69.

PARRISH, R.H., R. SERRA, and W.S. GRANT (1989). The Monotypic Sardines *Sardina* and *Sardinops*: Their Taxonomy, Distribution, Stock Structure and Zoogeography. Can. J. Fish. Aquat. Sci. **46**, 2019-2036.

PRAGER, E.M. and A.C. WILSON (1976). Congruency of Phylogenies Derived from Different Proteins. A Molecular Analysis of the Phylogenetic Position of Cracid Birds. J. Mol. Evol. **9**: 45-57.

RICHARDSON, B.J., BAVERSTOCK, P.R. and M. ADAMS (1986). Allozyme Electrophoresis. A handbook for Animal Systematics and Population Studies. Academic Press, Sydney. 410pp.

ROGERS, J.S. (1972). Measures of Genetic Similarity and Genetic Distance. Studies in Genetics, Univ. Texas Publ. 7213: 145-153.

RYMAN, N., LAGERCRANTZ, U., ANDERSSON, L., CHAKRABORTY, R., and R. ROSENBERG. (1984). Lack of Correspondence between Genetic and Morphologic Variability Patterns in Atlantic Herring (*Clupea harengus*). Heredity **53**(3): 687-704.

SCP. (1988). Economics and Marketing of Western Australian Pilchards. Fisheries Management Paper. Fish. Dept. West. Aust. No. **22**, 26p.

SEEB, L.W., SEEB, J.E., and POLOVINA, J.J. (1990). Genetic Variation in the Highly Exploited Spiny Lobster, *Panulirus marginatus*, Populations from the Hawaiian Archipelago. Fish. Bull. **88**(4): 713-718.

SHAKLEE, J.B., ALLENDORF, F.W., MORIZOT, D.C. and G.S.WHITT (1990a). Gene Nomenclature for Protein Coding Loci in Fish. Trans Am. Fish. Soc. **119**: 2-15.

SHAKLEE, J.B., PHELPS, S.R. and J. SALINI. (1990b). Analysis of Fish Stock Structure and Mixed-Stock Fisheries by the Electrophoretic Characterization of Allelic Isozymes. In Whitmore, D.H. (Ed.) "Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management." CRC Press. Boston.

SHAKLEE, J.B., BUSACK, C., MARSHALL, A., MILLER, M. and S.R. PHELPS (1990c). The Electrophoretic Analysis of Mixed-Stock Fisheries of Pacific Salmon. In: Z-O. Ogita and C.L. Markert, (eds.). Isozymes: Structure, Function and Use in Biology and Medicine. Progress in Clinical and Biological Research. **344**: 235-265. Wiley-Liss Inc. New York.

SHAW, C.R. and R. PRASARD (1970). Starch Gel Electrophoresis- a compilation of recipes. Biochem. Genet. **4**: 297-320.

SIN, F.Y.T and M.B. JONES (1983). Enzyme Variation in Marine and Estuarine Populations of a Mud Crab, *Macrophthalmus hiripes* (Ocypodidae). N. Z. J. Mar. Freshw. Res. **17**: 367-372.

SMITH, P.J. (1979). Esterase Gene Frequencies and Temperature Relationships in the New Zealand Snapper, *Chrysophrys auratus*. Marine Biology **53**:305-310.

SMOUSE, P.E., R.S. WAPLES, and J.A. TWOREK. (1990). A Genetic Mixture Analysis for use with Incomplete Source Population Data. Can. J. Fish. Aquat. Sci. **47**: 620-634.

STEPHENSON, R.L. and I. KORNFIELD (1990). Reappearance of Spawning Atlantic Herring(*Clupea harengus harengus*) on Georges Bank: Population Resurgence not Recolonization. Can. J. Fish. Aquat. Sci. **47**: 1060-1064.

SWOFFORD, D.L. and R.B. SELANDER (1989). BIOSYS-1. A Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systems. Release 1.7. Champaign, Illinois.

SYAHAILATUA, A. (1992). The Australian Pilchard (*Sardinops neopilchardus*); Morphometric, Meristic, Growth and Reproductive Studies. Unpublished M.Sc. Thesis. UNSW.

THOMPSON, D. and S. MOSTERT (1974). Muscle Esterase Genotypes in the Pilchard, *Sardinops ocellata*. J. Cons. int. Explor. Mer. **36**(1): 50-53.

WAPLES, R.S. (1990). Temporal Changes of Allele Frequency in Pacific Salmon: Implications for Mixed Stock Fishery Analysis. Can. J. Fish. Aquat. Sci. **47**: 968-976.

WAPLES, R.S., WINANS, G.A., UTTER, F.M. and C. MAHNKEN (1990). Genetic Approaches to the Management of Pacific Salmon. Fisheries **15**(5): 19-25.

APPENDICES

APPENDIX 1.0: Collection data for pilchards.

Key: ?= indeterminable; M= male; F= female; "non-spawning"= gonad maturity stages 1-3; "pre spawning"= stage 4; "spawning"= stages 5-7 (see Appendix 1.2); "mixed"=several individuals of spawning and non spawning status (assessment made by eye). += samples collected in the same month which were lumped for statistical analyses.

Table 1.1: Collection Data for Victoria

COLLECTION SITE Sample code	DATE	#INDIVID.	SEX RATIO F:M	SIZE RANGE (LCF mm)	BREEDING STATUS
Port Phillip Bay (PPB1)	21/02/91	100	55:44 ?=1	134.3-169.7	non spawning
Port Phillip Bay (PPB2)	25/03/92	94	46:46 ?=2	111-154	non spawning
Lakes Entrance (LKE1)	27/08/91	161	62:88 ?=11	80.1-190.3	non spawning

Table 1.2: Collection Data for New South Wales

COLLECTION SITE Sample code	DATE	#INDIVID.	SEX RATIO F:M	SIZE RANGE (LCF mm)	BREEDING STATUS
Wooli (not included in analyses)	18/05/90	124		111.5-143.6	?
Jervis Bay (JB1)	24/02/91	100	59:35 ?=6	120.6-150.2	non spawning
Jervis Bay (JB2)	03/03/92	96	38:56	122-158	non spawning

Table 1.3: Collection Data for South Australia

COLLECTION SITE Sample code	DATE	#INDIVID.	SEX RATIO F:M	SIZE RANGE (LCF mm)	BREEDING STATUS
Boston Bay (BSA1)	26/04/92	96	40:54 ?=2	128-174	non-prespawn

Table 1.4: Collection data for South Africa

COLLECTION SITE Sample code	DATE	#INDIVID.	SEX RATIO F:M	SIZE RANGE (LCF mm)	BREEDING STATUS
South Africa (SAF1)	??/01/91	32	??	??	??

Table 1.5: Collection Data for Western Australia

COLLECTION SITE Sample code	DATE	#INDIVID.	SEX RATIO F:M	SIZE RANGE (LCF mm)	BREEDING STATUS
Two People Bay	29/03/90	64	17:44 some spent	148.7-168.2	non spawning
Two People Bay	05/05/90	56	29:27	146.7-179.9	spawning
(both samples excluded from analysis, tissue was unreliable)					
Bremer Bay +	28/04/90	26	23:3	144.3-194	spawning
Bremer Bay (BB1)	29/04/90	24	21:3	145.8-167.9	spawning
Bremer Bay +	26/09/90	24	14:10	142-168.9	non spawning
Bremer Bay(BB2)	27/09/90	27	14:10	151-171.2	non spawning
Bremer Bay +	15/12/90	24	18:6	153-180	non-to-pre
Bremer Bay +	07/12/90	26	23:3	157-171	non-to-pre
Bremer Bay(BB3)	08/01/91	30	15:15	155-186	non spawning
Bremer Bay +	01/05/91	60	41:19	149-181	mixed
Bremer Bay(BB4)	08/06/91	65	50:17	146-172	mixed
Bremer Bay	19/12/91	72	51:21	141-183	non spawning
(Not analysed, denaturation of several enzymes had occurred.)					
Dunsborough (DUN1)	00/05/90	23	7:16	112.8-154.5	?
Dunsb. (DUN2)	22/11/90	84	45:39	130-175	spawning
Dunsb.+	20/12/90	46	33:13	139-193	spawning
Dunsb.(DUN3)	17/12/90	46	27:19	140-185	spawning
Dunsb. +	16/12/91	59	55:3	140-166	non-to-pre
Dunsb. (DUN4)	06/12/91	68	43:24 ?=1	137-171	non spawning
Esperance (ESP1)	10/05/90	63	43:20	145.9-180	spawning
Esperance +	14/11/90	14	6:8	156-170	non spawning
Esperance (ESP2)	01/11/90	45	14:26	138-168	non spawning
Esperance (ESP3)	10/05/91	22	too small	67.2-92.2	juveniles
Esperance (ESP4)	03/04/91	26	24:2	156-172	spawning
Esperance +	03/06/91	21	18:3	161-190	spawning
Esperance (ESP5)	05/06/91	25	17:8	156-180	mixed
Esperance +	16/01/92	44	38:6	128-163	non spawning
Esperance (ESP6)	19/01/92	32	18:14	149-171	non spawning
Fremantle (FM1)	11/05/90	47	19:28	146.3-162	non spawning
Fremantle +	31/01/91	11	4:2,?=5	133-146	non spawning
Fremantle (FM2)	16/01/91	18	12:M	130-165	non spawning
Fremantle (FM3)	16/05/91	114	69:44 ?=1	118-170	non spawning
Fremantle (FM4)	20/12/91	33	122:1	153-183	mixed

COLLECTION SITE Sample code	DATE	#INDIVID.	SEX RATIO F:M	SIZE RANGE (LCF mm)	BREEDING STATUS
King George Sound (KGS1)	30/03/90	46	17:28 ?=1	143.6-179.9	non spawning
King G.S.+	10/05/90	51	21:29 ?=1	116.9-183.8	pre spawning
King G.S. (KGS2)	07/05/90	33	20:13	163-183	spawning
King G.S. +	03/09/90	72	35:37	145-182	non spawning
KINGG.S. (KGS3)	06/09/90	66	32:34	137-172	non spawning
King G.S.+	05/01/91	34	21:13	149-177	pre spawning
King G.S.(KGS4)	12/01/91	25	19:6	161-184	pre spawning
King G.S. (KGS5)	05/04/91	82	65:17	153-178	pre-to-spawning
King G.S. +	10/05/91	48	18:30	148-177	mixed
King G.S. +	13/05/91	18	07:11	148-170	mixed
King G.S.(KGS6)	21/05/91	41	25:16	153-175	pre spawning
King G.S.	28/05/91	25	not electrophoresed		
King G.S.(KGS7)	06/12/91	110	50:60	153-180	mixed
Torbay (TOR1)	24/05/90	37	33:4	163.4-187.4	spawning
Torbay +	20/09/90	20	14:6	153.8-187.6	non spawning
Torbay (TOR2)	26/08/90	81	40:41	131.0-170.7	non spawning
Torbay +	05/12/90	20	14:6	158-179	spawning
Torbay +	18/01/91	25	21:4	163-185	spawning
Torbay (TOR3)	17/01/91	25	21:4	163-182	most spawning
Torbay (TOR4)	12/06/91	54	38:16	154-172	spawning
Cheynes Beach +	13/6/91	47	31:16	154-173	spawning
Ch. Beach (CHB1)	12/6/91	49	22:26	118-178	mixed

Appendix 1.2: Gonad maturity stages of Pilchards (Baker 1972).

Stage 1. INACTIVE: Small ovaries, either immature or mature, less than half the body cavity length, narrow but firm, pale pink; no eggs visible. Testes flat and leaf-like, pink or transparent.

Stage 2: INACTIVE/ACTIVE: Ovaries beginning to enlarge, slightly longer and up to 5mm thick, dark pink. Testes beginning to thicken and elongate, white colour developing.

Stage 3: ACTIVE: Ovaries longer than one half body cavity length, noticeably thicker, and yellow with pigmented eggs. Testes elongated to over half body cavity length, thickened, opaque white, with wavy edges.

Stage 4: ACTIVE/RIPE: Ovaries distended, almost completely filling body cavity, bright yellow, vascular; eggs discrete, becoming transparent at posterior end. Testes filling most of body cavity, opaque white, milkiness apparent at posterior end.

Stage 5: RIPE: Ovaries at maximum size, darker yellow and semi-transparent owing to even dispersal of ripe transparent eggs throughout gonads. Testes at maximum size, posterior half milky.

Stage 6: RIPE/RUNNING: The same as previous stage, but pressure on belly causes extrusion of eggs or milt.

Stage 7: SPENT: Ovaries elongated, but flat, hollow, and bloodshot; no large eggs present, except occasionally a few in oviduct. Testes elongated, strap-like, and bloodshot.

APPENDIX 2.0: Details of Buffers, Stains and Biochemicals used in their preparation

Appendix 2.1: Electrophoresis Buffer Recipes (Electrostarch)

TBE pH 90

Electrode Buffer:		3.96 g	Boric acid
		1.64 g	Na_2 EDTA
		38.75 g	Tris
	to	4.00 l	Milli Q water
Gel Buffer		as for electrode buffer	
Run Conditions		300V for 2h, 350V for next 3h.	

POULIK

Electrode Buffer:		76.4 g	Boric acid
		9.6 g	NaOH
	to	4.0 l	Milli Q water
Gel Buffer		37.2 g	Tris
		4.2 g	Citric acid
	to	4.0 l	Milli Q water
Run Conditions	Regulate on 35mA (voltage increases during run from 80V to 210V).		

TM pH 7.8

Electrode Buffer		24.2 g	Tris
		9.2 g	Maleic acid
	to	2.0 l	Milli Q water
Gel Buffer		100.0 ml	Electrode buffer
	to	1.0 l	Milli Q water
Run Conditions		50 mA, 200V, 3.5 hrs.	

CAM pH 6.1

Electrode Buffer		16.8 g	Citric acid
		19.5 ml	N-(3-aminopropyl)-morpholine
	to	2.0 l	Milli Q water
Gel Buffer		15.0 ml	Electrode buffer
	to	300.0 ml	Milli Q water
Run Conditions		50 mA, 190V, 3hrs.	

TC pH 5.8

Electrode Buffer		131.2 g	Tris
		84.1 g	Citric acid
		to 4.0 l	Milli Q water
Gel Buffer		70.0 ml	Electrode buffer
		to 2.0 l	Milli Q water
Run Conditions		50 mA, 200V, 3 hrs.	

TC pH 6.8

Electrode Buffer		64.17 g	Tris
		36.14 g	Citric acid
	to	2.00 l	Milli Q water
Gel Buffer		70.0 ml	Electrode buffer
		to 2.0 l	Milli Q water
Run Conditions		50 mA, 200V, 3 hrs.	

Appendix 2.2: Homogenizing Buffer Solution

NADP	50 mg
Mercaptoethanol	50 µl
Milli Q water	50 ml
Pyrodoxil 5 phosphate	5 mg

Appendix 2.3: Fixative Solution

Methanol : Acetic acid : Water Ratio 4 : 1 : 5

Appendix 2.4: Staining Buffer Recipes

0.2 M Na Citrate pH 4		8.4 g	Citric acid
	to	180.0 ml	Milli Q water
	titrate to	pH 4	with NaOH
	to final volume of	200.0 ml	Milli Q water
0.1 M Acetate pH 5		5.7 ml	Glacial acetic acid
	to	800.0 ml	Milli Q water
	titrate to	pH 5	with NaOH
	to final volume of	1.0 l	Milli Q water
0.1 M Phosphate pH 6.7		13.6 g	KH_2PO_4
	to	800.0 ml	Milli Q water
	titrate to	pH 6.7	with KOH
	to final volume of	1.0 l	Milli Q water
0.5 M Tris-HCl pH 7		121.1 g	Tris
	to	1.8 l	Milli Q water
	titrate to	pH 7	with conc. HCl.
	to final volume of	2.0 l	Milli Q water
0.1 M Phosphate pH 7.5		200.0 ml	0.5 M NaH_2PO_4
	to	800.0 ml	Milli Q water
	titrate to	pH 7.5	with NaOH
	to final volume of	1.0 l	Milli Q water
0.2 M Tris-HCl pH 8		48.4 g	Tris
	to	1.8 l	Milli Q water
	titrate to	pH 8	with conc. HCl
	to final volume of	2.0 l	Milli Q water
0.1 M Tris-HCl pH 8		24.2 g	Tris
	to	1.8 l	Milli Q water
	titrate to	pH 8	with conc. HCl
	to final volume of	2.0 l	Milli Q water

Appendix.2.5: Stain Recipes

Enzyme specific Histochemical Staining Recipes (modified from Harris and Hopkinson 1978 and Shaw and Prasard 1970)

ACONITATE HYDRATASE (AH)	**EC 4.2.1.3**
cis-Aconitate solution	20 ml
0.1 M $MgCl_2$	2 ml
NADP	0.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
Isocitrate dehydrogenase	5 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml
Aconitate Stock Solution	
cis-Aconitic acid	300 mg
TRIS	1 g
0.2 M Tris-Cl pH 8	80 ml
(Results in pH 8.1)	

ACID PHOSPHATASE (ACP)	**EC 3.1.3.2**
alpha-napthyl acid phosphate, Na salt	50 mg
0.1M Acetate pH 5.0	20 ml
Fast Garnett GBC salt (purified grade)	*10 mg*
2% Agar	*20 ml*

ADENOSINE DEAMINASE (ADA)	**EC 3.5.4.4**
Adenosine	20 mg
0.1 M Phosphate pH 7.5	15 ml
(Gently Heat)	
1M Na Arsenate	0.5 ml
Xanthine Oxidase	1 u
Nucleoside Phosphorylase	2 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

ALCOHOL DEHYDROGENASE (ADH)	**EC 1.1.1.1**
95% Ethanol	2 ml
0.2 M Tris-HCL pH 8	10 ml
NAD	8 ml
Na Pyruvate	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

ADENYLATE KINASE (AK)	**EC 2.7.4.3**
Glucose	100 mg
ADP	50 mg
0.2 M Tris-HCL pH 8	15 ml
0.1 M $MgCl_2$	1 ml
NADP	2.5 ml
Na Pyruvate	1 ml
Pyrazole	1 ml
Hexokinase	100 u
G-6-P-DH	60 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

ALDOLASE (ALD) **EC 4.1.2.13**

Fructose 1,6 di-Phosphate	100 mg
0.5 M Tris-HCL pH 7	20 ml
1M Na Arsenate	0.4 ml
NAD	8 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
Triosephos. isomerase	50 u
Glyeraldehyde-3-phos.-DH	50 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

ALDEHYDE DEHYDROGENASE (ALDH) EC 1.2.1.3

0.2 TRIS HCl, pH 8.0	10 ml
Benzaldehyde	2 ml
NAD	4 ml
1M KCl	4 ml
Na pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% Agar	20 ml

ALKALINE PHOSPHATASE (ALP) EC 3.1.3.1

Beta-Naphthyl Phosphate	25 mg
0.2 M Tris-HCl pH 8	20 ml
$MgSO_4$/KCl	0.5 ml
Fast Garnet GBC Salt (purified grade)	10 mg
2% AGAR	20 ml

ALDEHYDE OXIDASE (AO) **EC 1.2.3.1**

0.2M Tris -HCL pH8	20 ml
Benzaldehyde	0.2 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

ASPARTATE AMINOTRANSFERASE (AAT) EC 2.6.1.1

AAT Substrate Solution	20 ml
Fast Blue BB Salt (purified grade)	10 mg
2% AGAR	20 ml

Aspartate Aminotransferase Substrate Solution

alpha-Ketoglutaric Acid		0.29 g
L-Aspartic Acid		1.06 g
Polyvinylpyrrolidone		4.00 g
Na_2 EDTA		0.40 g
Na_2 H PO_4		11.36 g
H_2O	to	400 ml

ASPARTATE AMINOTRANSFERASE (AAT) EC 2.6.1.1 (alternative recipe)

L-Cysteine Sulfinic Acid	40 mg
Pyridoxal-5'-Phosphate	10 mg
alpha-Ketoglutaric Acid	40 mg
0.2M TRIS-HCl pH8	20 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

CREATINE KINASE (CK) EC 2.7.3.2

Creatine Phosphate	20 mg
ADP	50 mg
Glucose	45 mg
0.5 M Tris-HCL pH 7	15 ml
NADP	1.5 ml
0.1M $MgCl_2$	0.5 ml
Hexokinase	160 u
G-6-P-DH	80 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

D-AMINO ACID OXIDASE (DAMOX) EC 1.4.3.3

D-amino acid (eg. D-methionine)	200 mg
0.2M Tris-HCL pH 8	20 ml
(Adjust to pH 8 with unbuffered 2M Tris if necessary)	
FAD	10 mg
Peroxidase	10 mg
3-amino-9-ethyl carbazole	1 ml
2% AGAR	20 ml

DIAPHORASE (DIA) EC 1.6.2.2

0.2 M Tris-HCL pH 8	10 ml
NADH	30 mg
MTT	1 ml
2,6-dichlorophenol	0.75 ml
Water to	50 ml

ESTERASE (EST) EC 3.1.1.1

(Carboxylesterase)

0.1 M Phosphate pH 6.7	10 ml
Esterase Substrate Solution	1.5 ml
(allow to reach R.T before use)	
Fast Garnet GBC Salt (purified grade)	10 mg
2% AGAR	20 ml

Esterase Substrate Solution

Alpha-Naphthyl Acetate	0.5 g
Beta-Naphthyl Acetate	0.5 g
Acetone	25 ml
H_2O to	50 ml

FRUCTOSE-bisPHOSPHATASE (FBP) EC 3.1.3.11

Fructose-1,6-diPhosphate	50 mg
0.2 M Tris-HCl pH 8	20 ml
0.1 M $MgCl_2$	0.5 ml
NADP	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
Phosphoglucose Isomerase	50 u
Glucose-6-Phosphate DH	30 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

FUMARATE HYDRATASE (FH) **EC 4.2.1.2**

(Fumarate Hydratase)

Fumaric acid	100 mg
0.5 M Tris-HCL pH 7	20 ml
NAD	4 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MDH	100 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

GALACTOSE DEHYDROGENASE (GALDH) EC 1.1.1.48

Galactose	500 mg
0.2 M Tris-HCl pH 8	20 ml
NAD	5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

GLUCONATE-5-DEHYDROGENASE (GDH) EC 1.1.1.69

D-Gluconate (Na salt)	50 mg
0.2 M Tris-HCl pH 8	20 ml
NADP	1 ml
0.1 M $MgCl_2$	0.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% Agar	20 ml

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH) EC 1.1.1.49

0.2 M Tris-HCL pH 8	10 ml
0.25 M Glucose-6-phosphate	3 ml
NADP	1 ml
0.1 M $MgCl_2$	0.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2 % AGAR	20 ml

alpha-GLUCOSIDASE (aGLU) **EC 3.2.1.20**

Maltose	50 mg
0.1 M Acetate pH 5	20 ml
Peroxidase	10 mg
Glucose Oxidase	50 u
o-Dianisidine	0.4 ml
2% AGAR	20 ml

GLUCOSEPHOSPHATE ISOMERASE (GPI) EC 5.3.1.9

(Glucose-6-phosphate Isomerase)

Fructose-6-phosphate	40 mg
0.2 M Tris-HCL pH 8	15 ml
NADP	0.2 ml
0.1 M $MgCl_2$	0.1 ml
Glucose-6-Phosphate DH	10 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

GLUTAMATE DEHYDROGENASE (GLUDH) EC 1.4.1.3

Na Glutamate	70 mg
0.2 M Tris-HCL pH 8	20 ml
NADP	0.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2 % AGAR	20 ml

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GA3PDH) EC 1.2.1.12

To prepare substrate:

Fructose-1,6-Diphosphate	50 mg
0.2 M Tris-HCl pH 8	2 ml
Aldolase	5 ul

Incubate at 37°C for 1 hour

Then Add:

0.2 M Tris-HCl pH8	20 ml
NAD	3 ml
1 M Na-Arsenate	0.2 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

GLYCEROL DEHYDROGENASE (GLYDH) EC 1.1.1.6

0.2 M Tris pH8	20 ml
0.1 M Glycerol	5 ml
NAD	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

alpha-GLYCEROPHOSPHATE DEHYDROGENASE (GPD) EC 1.1.1.8

Na glycerophosphate	300 mg
0.2 M Tris-HCl pH 8	20 ml
NAD	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

GLYCOLATE OXIDASE (GOX) **EC 1.1.3.15**
((S)-2-Hydroxy-acid Oxidase)

Glycolic Acid	1 ml
0.2 M Tris-HCl pH 8	20 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

GLYCOLATE OXIDASE (GOX) **EC 1.1.3.1**
(Alternative Recipe)

alpha-Hydroxyisocaproic acid	25 mg
0.2 M Tris-HCl pH 8	20 ml
Peroxidase	10 mg
o-Dianisidine	0.4 ml
2% AGAR	20 ml

GLYOXALASE II (GLO II) **EC 3.1.2.6**
(Hydroxyacylglutathione Hydrolase)

Glutathione (oxidised)	40 mg
0.1 M Tris-HCL pH 8	15 ml
NAD	4 ml
Methylglyoxal	50 ul
Pyrazole	1 ml
Glo I	50 u
LDH	30u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	15 ml

GUANINE DEAMINASE (GDA) **EC 3.5.4.3**

0.2 M Tris-HCL pH 8	20 ml
Guanine Substrate Solution	3 ml
MTT	0.5 ml
PMS	0.2 ml
Xanthine oxidase	10 u
2% AGAR	20 ml

Guanine Substrate Solution

Guanine	50 mg
1 M NaOH	5 ml
(gently heat)	
H_2O to	50 ml

HEXOKINASE (HK) **EC 2.7.1.1**
(ß-N-Acetylhexosaminidase)

Glucose	50 mg
ATP	40 mg
0.5 M Tris-HCL pH 7	10 ml
0.1 M $MgCl_2$	0.5 ml
NADP	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
G-6-PDH	20 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

HEXOSAMINIDASE (HEX) EC 3.2.1.52

(ß-N-Acetylglucosaminidase)

NAG (Naphthol-AS-BI-2-acetamido-2-deoxy-B-D-glucopyranoside)	20 mg
Methanol (Absolute)	10 ml
(Gently Heat)	
0.1 M Acetate pH 5.0	20 ml
Fast Garnett GBC Salt (Purified Grade)	10 mg
2% AGAR	20 ml

3-HYDROXYBUTYRATE DEHYDROGENASE (HBDH) EC 1.1.1.30

DL-ß-Hydroxybutyric Acid	600 mg
NaCl	300 mg
0.5 M Tris-HCL pH 7	20 ml
NAD	3 ml
Na Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

ISOCITRATE DEHYDROGENASE (IDHP) EC 1.1.1.42

DL-Isocitrate	5 ml
0.2 M Tris-HCL pH 8	20 ml
0.1 M $MgCl_2$	0.5 ml
NADP	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

LACTATE DEHYDROGENASE (LDH) EC.1.1.1.27

0.2 M Tris-HCL pH 8	10 ml
70 % Na-Lactate	2 ml
NAD	2.5 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

MALATE DEHYDROGENASE (MDH) EC 1.1.1.37

0.5 M Tris-HCL pH 7	15 ml
1 M Na-Malate	5 ml
NAD	2.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

Na-L-Malate Substrate Solution

Na_2CO_3		24.3 g
L-Malic acid		26.8 g
H_2O	to	200 ml

MALIC ENZYME (MEP) EC 1.1.1.40

0.5 M Tris-HCL pH 7	15 ml
1M Na-Malate	5 ml
NADP (solid)	15 mg
0.1 M $MgCl_2$	0.5 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

MANNITOL DEHYDROGENASE (MADH) EC 1.1.1.67

D-Mannitol	50 mg
0.2 M Tris-HCl pH 8	20 ml
NAD	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

MANNOSE PHOSPHATE ISOMERASE (MPI) EC 5.3.1.8

0.2 M Tris-HCL pH 8	10 ml
Mannose-6-phosphate	20 mg
NADP	1 ml
$MgCl_2$	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
Phosphoglucoseisomerase	80 u
Glucose-6-Phosphate DH	60 u
MTT	0.5 ml
PMS	0.2 ml
2 % AGAR	20 ml

PHOSPHOGLUCOMUTASE (PGM) EC 5.4.2.2

0.5 M Tris-HCL pH 7	15 ml
5% Glucose-1-Phosphate	3 ml
0.1 M $MgCl_2$	0.5 ml
NADP	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
Glucose-6-Phosphate DH	20 u
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

PEPTIDASE (PEP) EC 3.4.11. or 3.4.13.9

0.1 M Phosphate pH 7.5	15 ml
Peptide*	20 mg
0.1 M $MgCl_2$	0.5 ml
Peroxidase	10 mg
Amino Acid Oxidase	5 mg
O-Dianisidine HCL	0.5 ml
2 % AGAR	20 ml

* For Pep A & C: leu-ala
Pep B: leu-gly-gly; Pep D: leu-pro or phe-pro
Pep S,B,E,F: leu-leu-leu. Also try leu-tyr, lys-leu,val-leu, etc.

PHOSPHOGLUCONATE DEHYDROGENASE (PGDH) EC 1.1.1.44

0.2 M Tris-HCL pH 8	10 ml
6-Phosphogluconic acid	20 mg
NADP	1 ml
0.1 M $MgCl_2$	0.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2 % AGAR	20 ml

SUCCINATE DEHYDROGENASE (SUCDH) EC 1.3.99.1

0.1M Phosphate pH 7.5	15 ml
Na-Succinate	100 mg
FAD	10 mg
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

XANTHINE DEHYDROGENASE (XDH) EC 1.1.1.204

0.5 M Tris-HCl pH7	20 ml
Hypoxanthine	50 mg
Just before slicing gel:	
Bring to the boil to dissolve hypoxanthine.	
Cool to R.T.	
NAD	2.5 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

Appendix 2.6: Stock Solutions Used in Enzyme-Specific Stain Recipes

SOLUTION	CONCENTRATION
Acetoacetyl CoA	2.5 mg/ml
o-Dianisidine	10 mg/ml
2,6-Dichlorophenol	5mg/ml
Glucose-6-phosphate Dehydrogenase	10u/ml
$MgCl_2$	2g/100ml
$MgCl_2$/KCl	1g each/25ml
MTT	10mg/1.5ml
NAD	1g/100ml
NADP	1g/100ml
Na-Arsenate	18.6g/100ml
Na-Pyruvate	5g/100ml
Phosphoglucoseisomerase	10u/ml
PMS	10mg/ml
Pyrazole	5g/100ml

APPENDIX 3.0: Pilot Study of the Australian Pilchard.

Appendix 3.1: Enzymes investigated in the Australian pilchard

ENZYME	ABBREVIATION	ENZYME COMMISSION #
Aspartate aminotransferase	AAT	EC 2.6.1.1
Acid phosphatase	ACP	EC 3.1.3.2
Aconitate hydratase	AH	EC 4.2.1.3
Adenosine deaminase	ADA	EC 3.5.4.4
Adenylate kinase	AK	EC 2.7.4.3
Alcohol dehydrogenase	ADH	EC 1.1.1.1
Aldolase	ALD	EC 4.1.2.13
Aldehyde dehydrogenase	ALDH	EC 1.2.1.3
Aldehyde oxidase	AO	EC 1.2.3.1
Alkaline phosphatase	ALP	EC 3.1.3.1
Creatine kinase	CK	EC 2.7.3.2
D-Amino acid oxidase	DAMOX	EC 1.4.3.3
Diaphorase	DIA	EC 1.8.1.4
Esterase	EST	EC 3.1.1.1
Fructose-bisphosphatase	FBP	EC 3.1.3.11
Fumarate hydratase (Fumarase)	FH	EC 4.2.1.2
Galactose dehydrogenase	GALDH	EC 1.1.1.48
Gluconate-5-dehydrogenase	GDH	EC 1.1.1.69
Glucose-6-phosphate dehydrogenase	G6PDH	EC 1.1.1.49
Glucose-6-phosphate isomerase	GPI	EC 5.3.1.9
alpha-Glucosidase	aGLU	EC 3.2.1.20
Glutamate dehydrogenase	GLUDH	EC 1.4.1.3
Glyceraldehyde-3-phosphate dehydrogenase	GA3PDH	EC 1.2.1.12
Glycerol dehydrogenase	GLYDH	EC 1.1.1.6
alpha-Glycerophosphate dehydrogenase	GPD	EC 1.1.1.8
Glycolate oxidase	GOX	EC 1.1.3.15
Glyoxalase II	GLO II	EC 3.1.2.6
Guanine deaminase	GDA	EC 3.5.4.3
Hexokinase	HK	EC 2.7.1.1
Hexosaminidase	HEX	EC 3.2.1.52
beta-Hydroxybutyrate dehydrogenase	HBDH	EC 1.1.1.30
Isocitrate dehydrogenase	IDHP	EC 1.1.1.42
Lactate dehydrogenase	LDH	EC 1.1.1.27
Malate dehydrogenase	MDH	EC 1.1.1.37
Mannitol dehydrogenase	MADH	EC 1.1.1.67
Malic enzyme	MEP	EC 1.1.1.40
Mannose phosphate isomerase	MPI	EC 5.3.1.8
Phosphoglucomutase	PGM	EC 5.4.2.2
Peptidases	PEP	EC 3.4.11 or 13 or 3.4.13.9
Phosphogluconate dehydrogenase	PGDH	EC 1.1.1.44
Succinate dehydrogenase	SUCDH	EC 1.3.99.1
Xanthine dehydrogenase	XDH	EC 1.1.1.204

Appendix 3.2: Enzymes studied, tissues investigated, electrophoresis running conditions and presumed number of loci for pilchard pilot study.

Key: **L** = liver, **M** = muscle, **H** = heart.

* = best tissue/buffer/support matrix for this enzyme,

1 = Tris- EDTA-boric acid pH 9, **2** = Poulik, **3** = Tris-maleate pH 7.8,

4 = Tris-citric acid pH 6.8, **5** = Tris-citric acid pH 5.8,

6 = Citric acid-aminopropyl-morpholine pH 6.1,

C = cathodal; **A** = anodal;

ST = Electrostarch gel

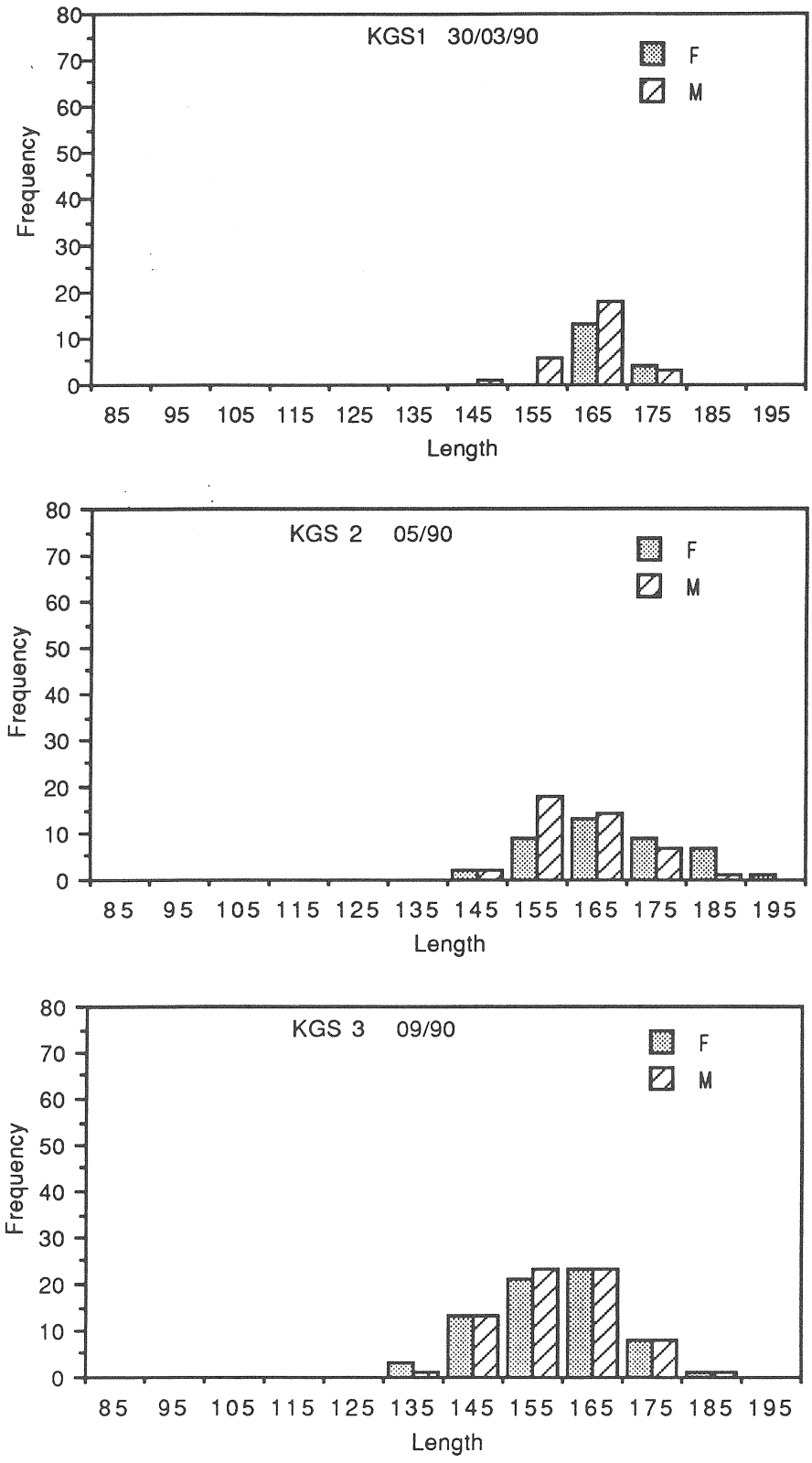
P = polymorphic.

Enzyme	Tissue	Buffer(s)	Presumed # loci	Comments
AAT	L	2*,3*,6	1A	fair activity, poor resolution P
	M	1,4,6	1A	poor resolution
	H	2,3,5,6	1A	fair activity
AAT (altern)	L	3*,5*	2A	fast staining, good activity, P
AH	L	1,2,3,4,6*	1A	good activity, P
	M	1,2,3,4,6	1A	fair activity
	H	1,2,3,4,6	1A	good activity, P
ACP	L	1,2,5.	2A	fair resolution
	M	1,3,4,5*,6	2A	fair resolution, good activity
	H	1,2,5	2A	fair resolution
ADA	L	2,3,6		no activity
	M	2,3,6		no activity
	H	2,3,6		no activity
ADH	L	1,2,3,4,6	1A	poor activity, fair resolution
	M	1,4,5,6	1A	no activity
	H	2,3,5,6	1A	no activity
AK	L	1,2,3,4,5,6	1	good activity; poor resolution
	M	1,2,3,4,5,6	1	good activity
	H	1,2,3,4,5,6*	1	good activity, fair resolution
ALD	L	1,2,3,6	1C	good activity; poor resolution
	M	1,3,4,6*	1C	good activity, fair resolution
	H	1,2	1C	good activity, poor resolution
ALDH	L	1,2,3,4,5,6	1	poor activity
	M	2,3,6	1	poor activity
	H	1*,4,5	1	good activity, good resolution
ALP	L	2,3,6	1	poor activity
	M	2,3,6	1	poor activity
	H	2,3,6	1	poor activity
AO	L	1,2,5.	1	poor activity
	M	1,3,4,5,6		no activity
	H	12,3,5,6		no activity
CK	L	2,3,4,6	2A	good activity, poor resolution
	M	2,3,4,6*	2A	good activity, fair resolution
	H	2,3,4,6	2A	good activity, poor resolution
DAMOX	L	5 *	1A	poor activity
	M	5	1A	poor activity
	H	5	1A	poor activity
DIA	L	1*,2,3,4,5,6	1A	fair activity, fair resolution
	M	2,3,6	1A	poor activity

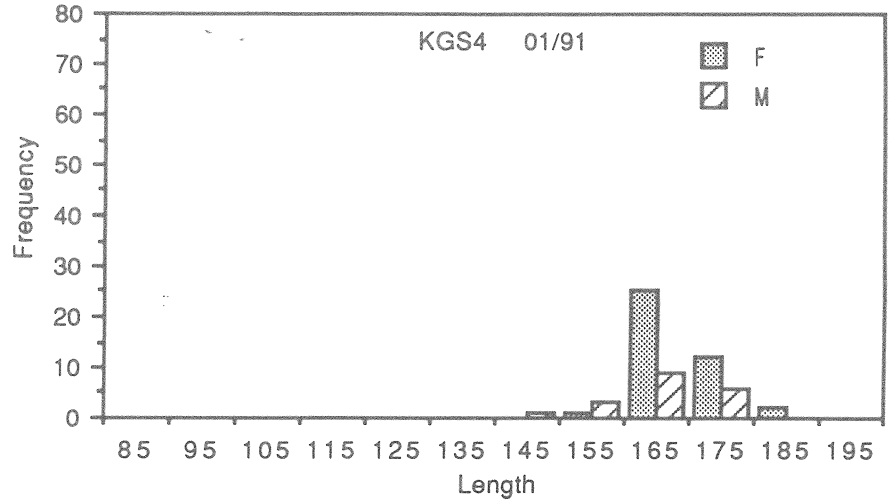
Enzyme	Tissue	Buffer(s)	Presumed # loci	Comments
EST	L	1,2*,3*,4,5,6	4A	good activity,good resolution, P
	M	2,3,5	4A	poor activity
	H	1,4,6	4A	poor activity
FBP	L	2,3,4,6*	1A	fair activity, sub-banding
	M	2,3,4,6	1A	poor activity
	H	2,3,4,6	1A	poor activity
FH	L	1,2,3,4,5,6	1A	poor activity
	M	1,2,3,4,5	1A	poor activity
	H	1,2*,3,4,5,6	1A	good activity, good resolution
G6PDH	L	1,6.	1A	poor activity
	M	1,2,3,6	1A	poor activity
	H	1,2,3,6*	1A	good activity, fair resolution
GA3PDH	L	6	1C	poor activity
	M	6	1C	good activity, fair resolution
	H	6	1C	poor activity
GALDH	L	1,2,3,4,5,6	1A	poor activity, poor resolution
	M	1,2,3,4,5,6		no activity
	H	1,2,3,4,5,6		no activity
GDA	L	1,2,3,4,5,6	1	poor activity
	M	1,6		no activity
	H	1,2,3,4,5,6		no activity
GDH	L	1,2,3,4,5,6		no activity
	M	1,2,3,4,5,6		no activity
	H	1,2,3,4,5,6		no activity
GLO II	L	2,3*,6	1A	good activity, good resolution
	M	-	1A	-
	H	1,2,4*,5	1A	good resolution
aGLU	L	6	1A	poor activity
	M	6		no activity
	H	6		poor activity
GLUDH	L	1,2		no activity
	M	3,4,6		no activity
	H	1,2,3,4,5,6		no activity
GLYDH	L	1,2,3,4,5,6		no activity
	M	1,2,3,4,5,6		no activity
	H	1,2,3,4,5,6		no activity
GOX	L	1,2		no activity
	M	3,4,6		no activity
	H	1,2,3,4,5,6		no activity
GPD	L	1,2,3,4*,5,6	1A	good activity, good resolution
	M	2,3,6		no activity
	H	1,2,3,4,5,6		no activity
GPI	L	1,2,3,4*,5,6	1A	good activity, sub-banding
	M	1,2,6	1A	poor activity
	H	1,2,3*,4,5,6	1A	good activity, fair resolution
HBDH	L	2,3,6		no activity
	M	-		-
	H	-		-
HEX	L			
	M	1,2,3.		no activity
	H	1,2,3.		no activity
HK	L	2,3,4,6*	1A	fair activity, fair resolution
	M	2,3,4,6		no activity
	H	1,2,3,4,5,6	1A	fair activity

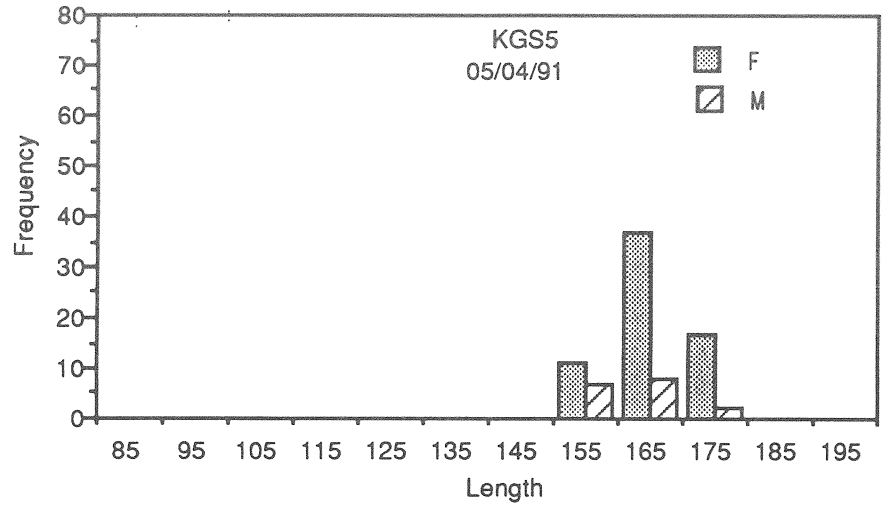
Enzyme	Tissue	Buffer(s)	Presumed # loci	Comments
IDHP	L	2,3,5	2A	poor activity
	M	1,2,3,4,6	2A	fair activity
	H	1,2,3,4,5*,6*	2A	good activity, fair resolution
LDH	L	3	1A	good activity, sub-banding
MADH	L	3		no activity
MDH	L	1,2,3,4,5,6	1A	good activity, sub-banding
	M	1,2,3,4,5,6*	1A	good activity, fair resolution
	H	1,4.	1A	good activity
MEP	L	1,2,3,4,5*,6	1A	good activity, fair resolution
	M	1,2,3,4,5,6	1A	fair activity
	H	1,2,3,4,5,6*	1A	good activity, fair resolution
MPI	L	1,2,3,4,5*,6	1A	fair activity, P
	M	1,2,3,4,5,6		no activity
	H	1,2,3,4,5,6*	1A	fair activity, P
PEPA	L	3,6*	2A	good activity, sub-banding
	M	6	2A	good activity, good resolution
	H	6	2A	good activty
PEPB	L	6*	1A	good activity, good resolution,P
	M	6	1A	poor activity
	H	6	1A	poor activity
PEP (leu.leu.leu)	L	6	2A	good activity, fair resolution P
	M	6	2A	poor activity
	H	6		no activity
PEPD	L	6	1	fair activity, poor resolution
	M	6	1	fair activity
	H	6	1	poor activity
PEP (leu-tyr)	L	1	1A	fair activity, fair resolution
PEPS	L	6	1A	poor activity
	M	6	1A	poor activity
	H	6		no activity
PGD	L	1,3,6	1A	poor activity, poor resolution
	M	6	1A	poor activity
	H	6	1A	poor activity
PGM	L	1,2,3*,4,5,6*	1A	good activity, good resolution, P
	M	1*,2,6	1A	good activity, poor resolution
	H	1,2,3,4,5,6	1A	good activity
SUCDH	L	1,2,3,4,5,6		no activity
	M	1,2,3,4,5,6		no activity
	H	1,2,3,4,5,6	1A	poor activity
XDH	L	3		no activity

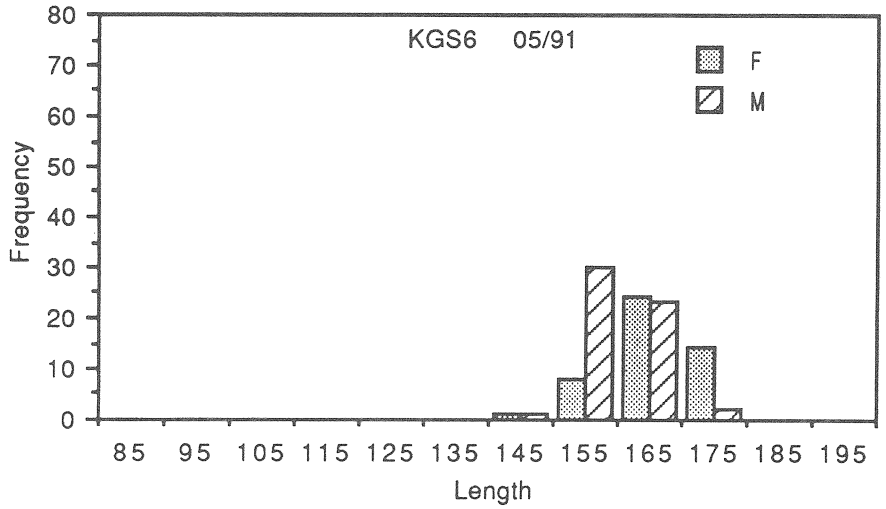
APPENDIX 4.0 Length frequency histograms for all samples



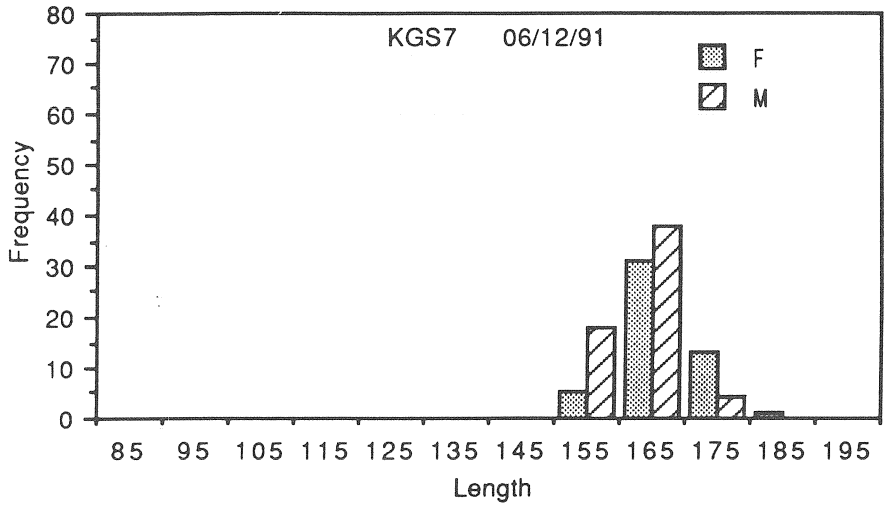
Appendix 4.1: Length frequency histograms of King George Sound samples, WA



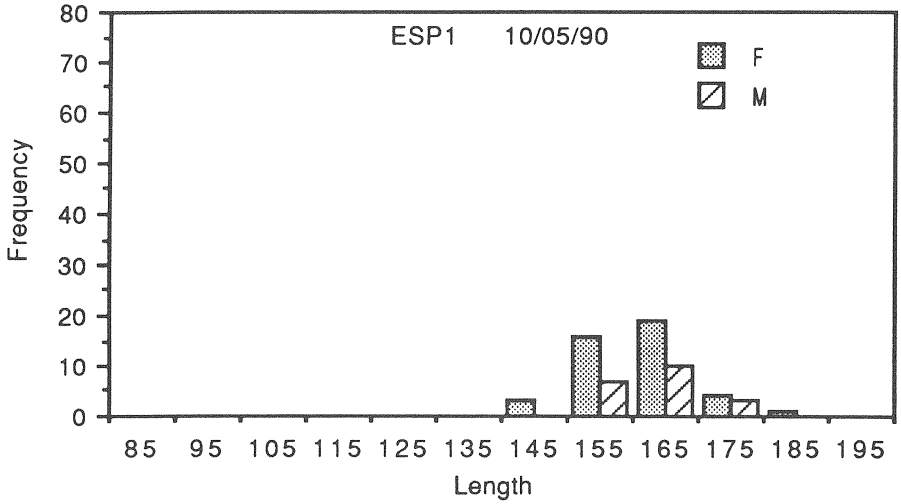


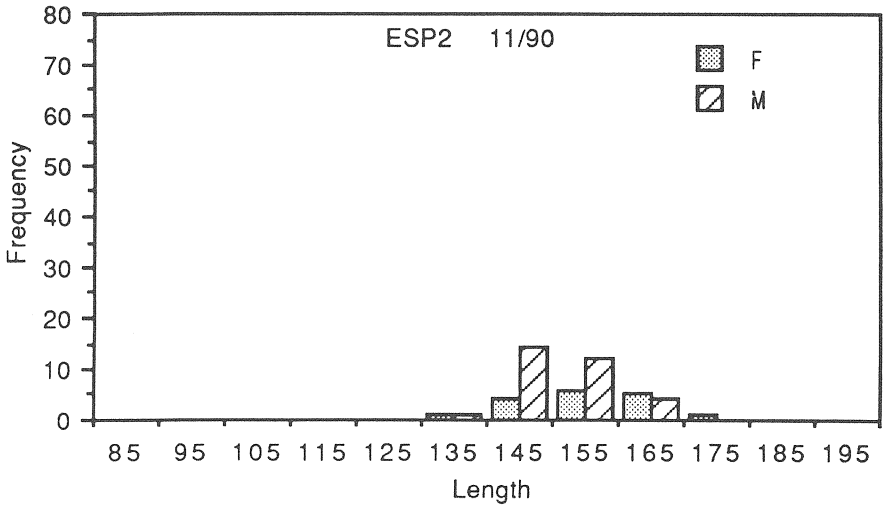


Appendix 4.1: Length frequency histograms for King George Sound samples, WA

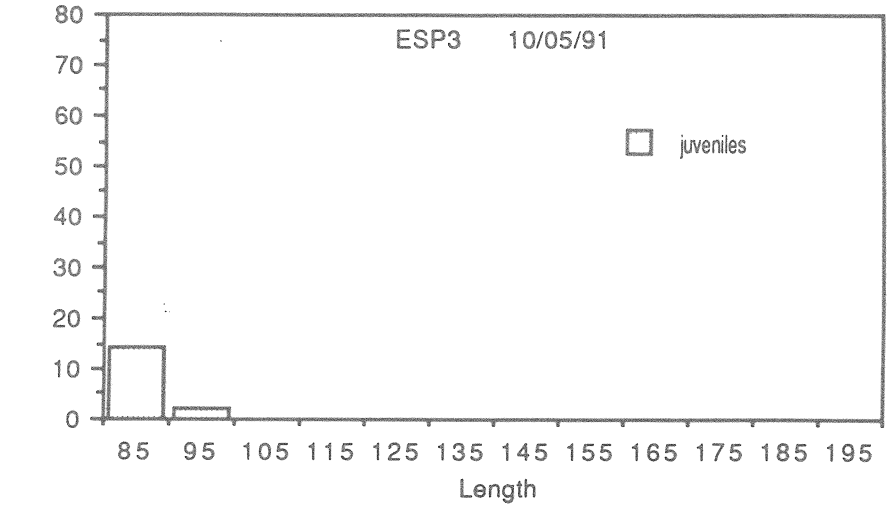


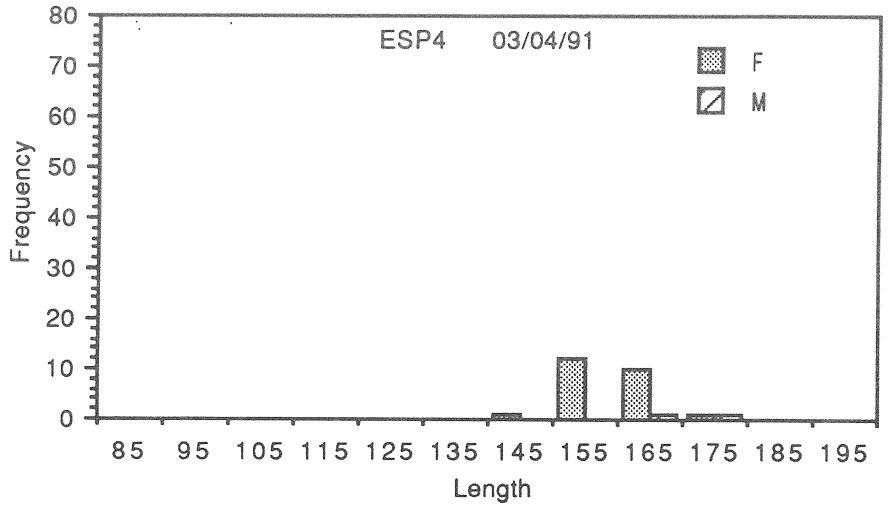
Appendix 4.1: Length frequency histograms for King George Sound samples, WA

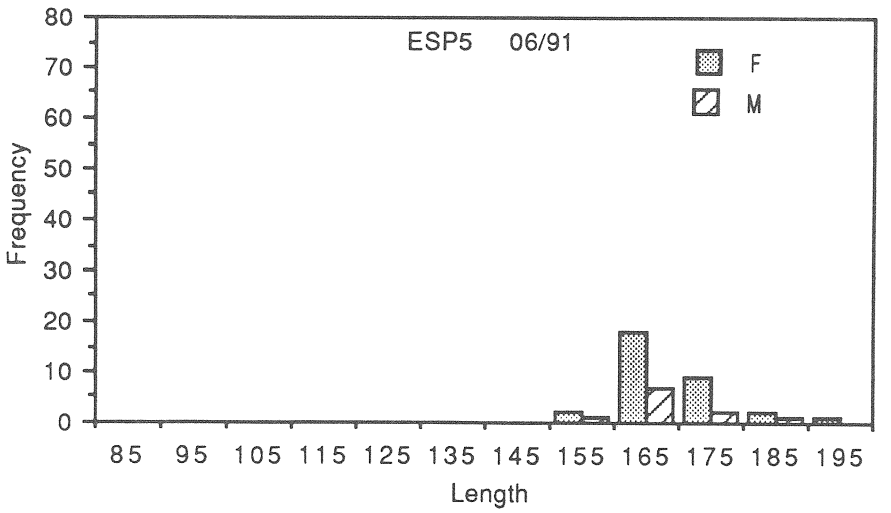




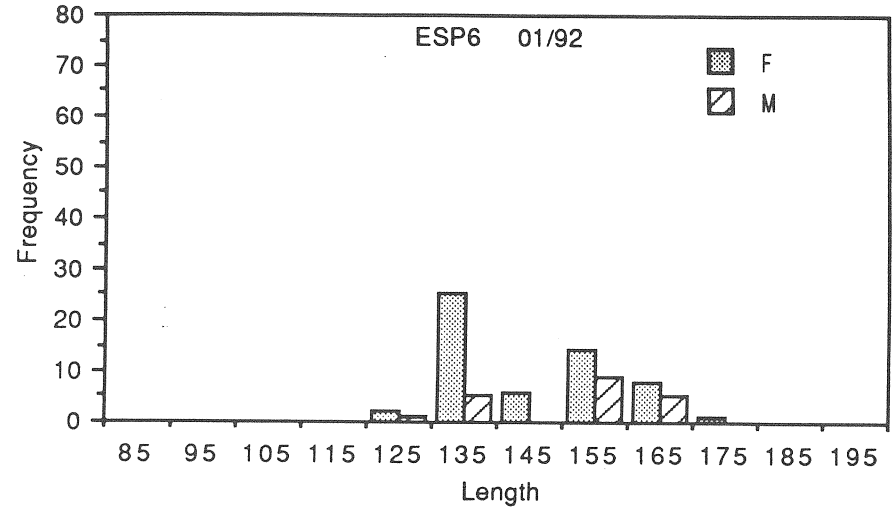
Appendix 4.2: Length frequency histograms for Esperance samples, WA



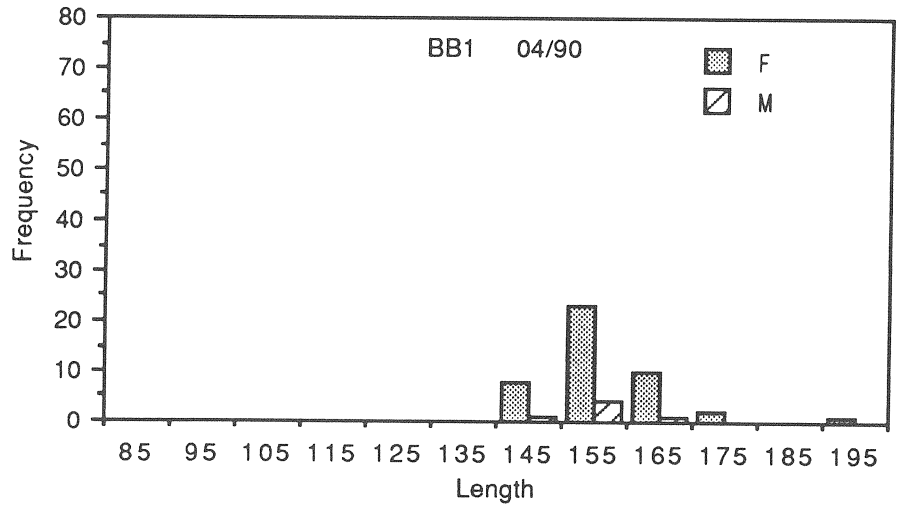


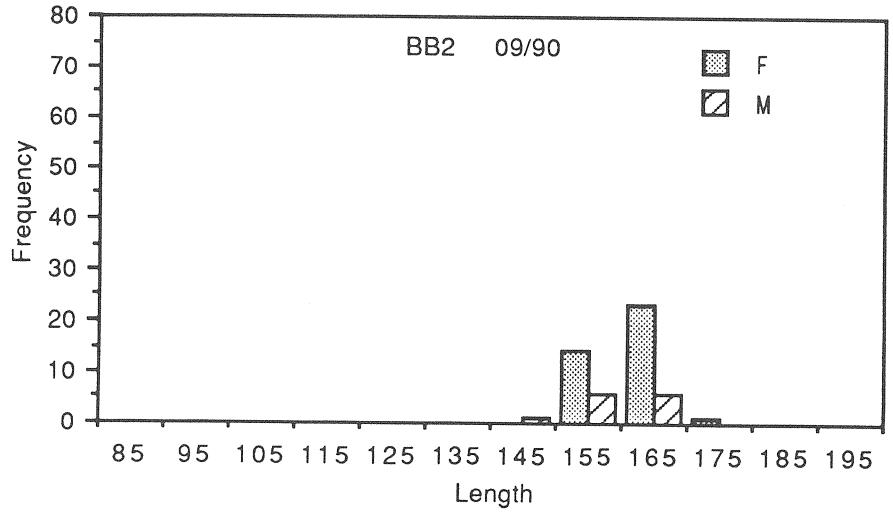


Appendix 4.2: Length frequency histograms for Esperance samples, WA

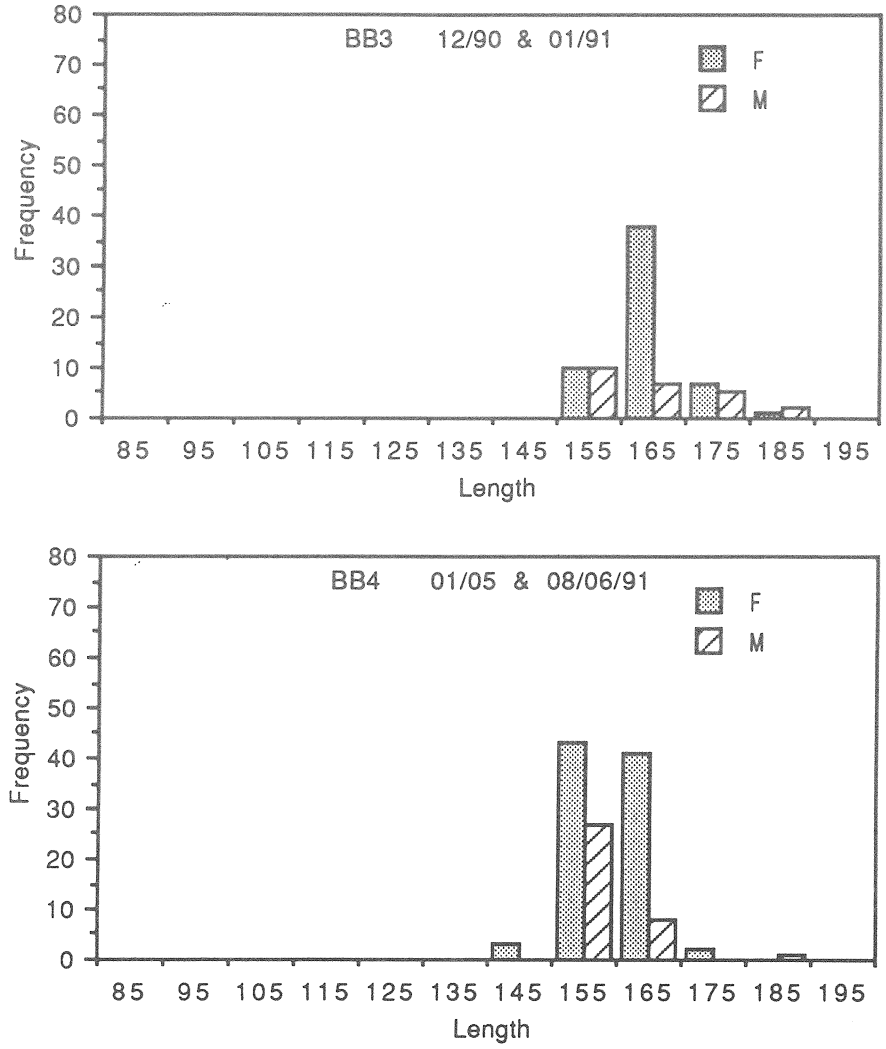


Appendix 4.2: Length frequency histograms for Esperance samples, WA

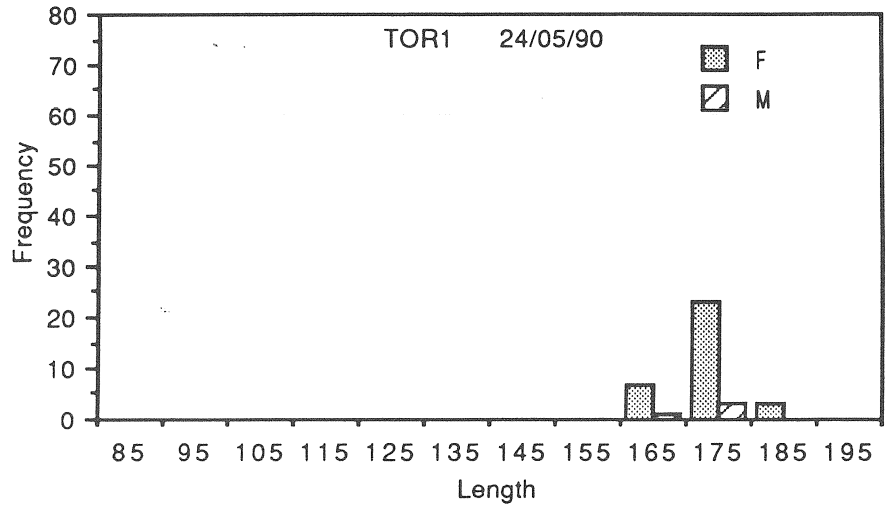


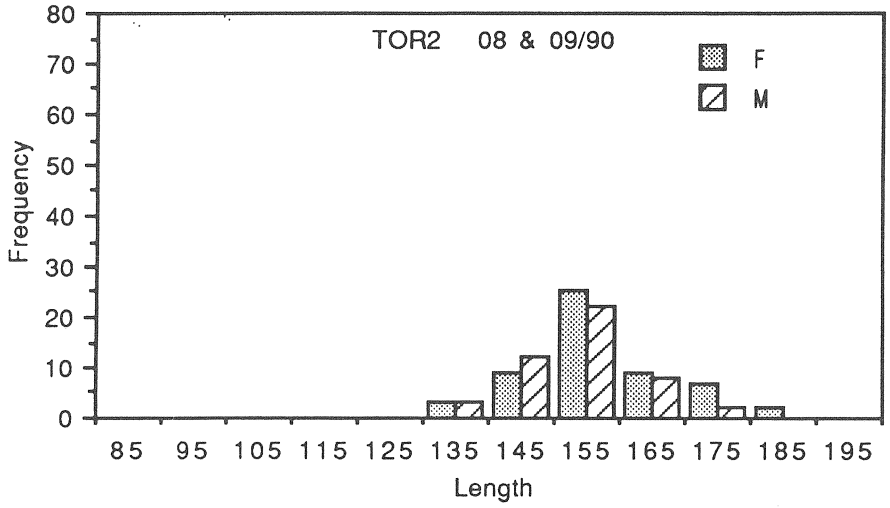


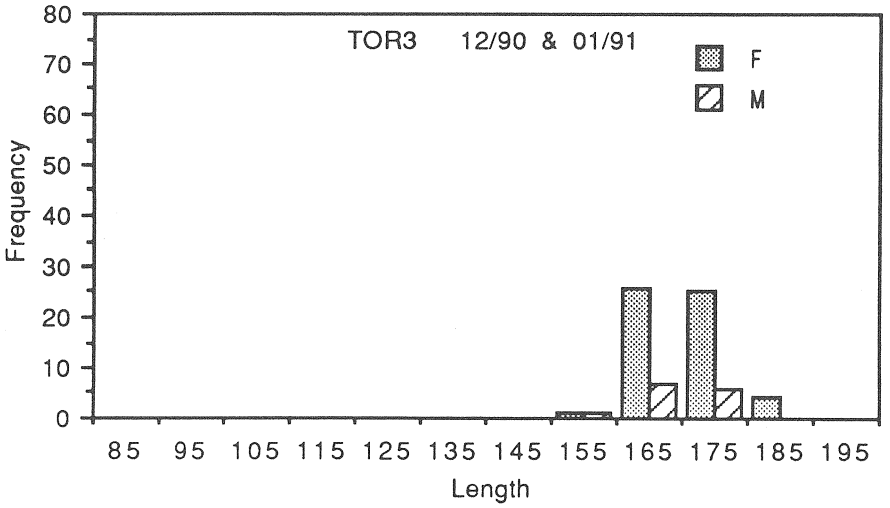
Appendix 4.3: Length frequency histograms for Bremer Bay samples, WA



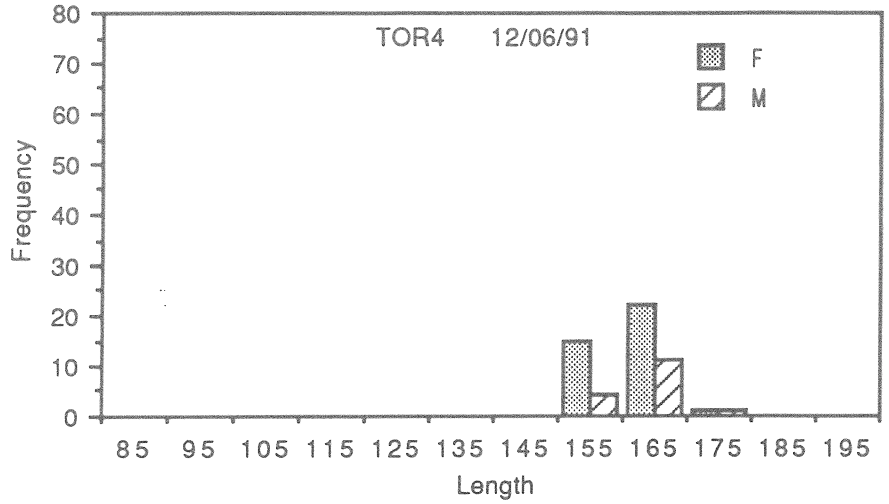
Appendix 4.3: Length frequency histograms for Bremer Bay samples, WA



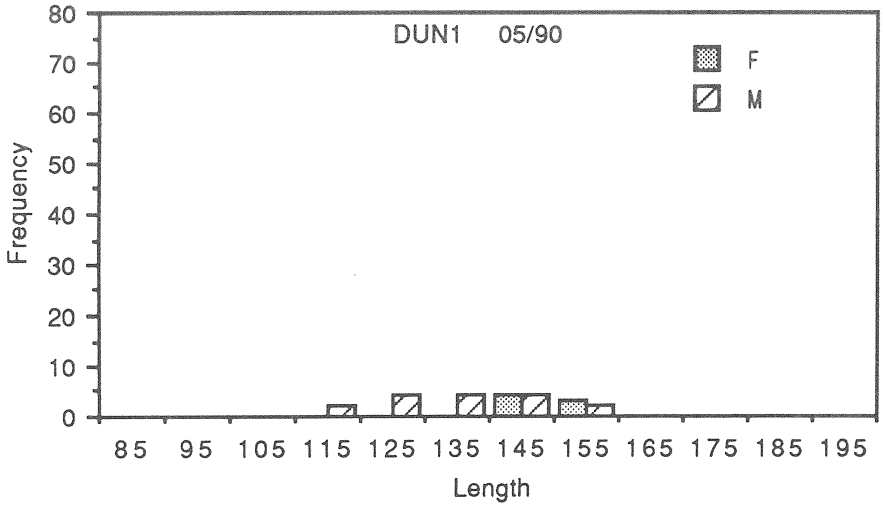


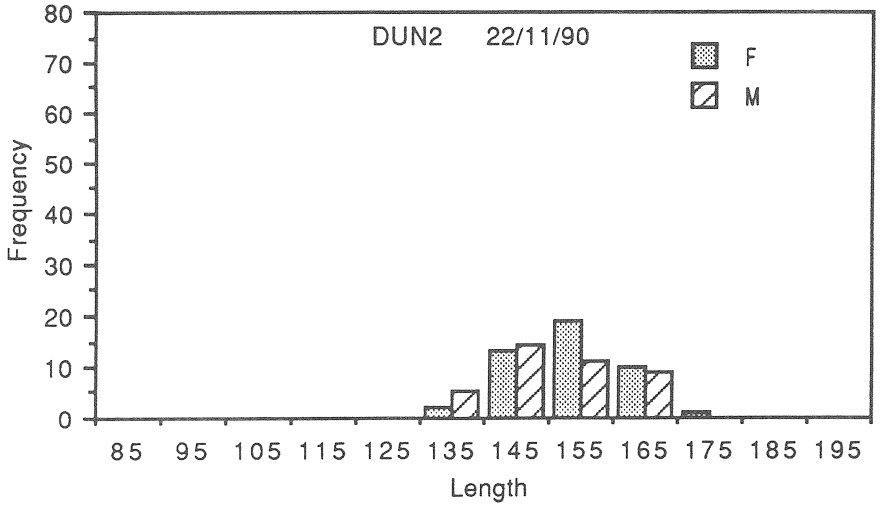


Appendix 4.4: Length frequency histograms for Torbay samples, WA

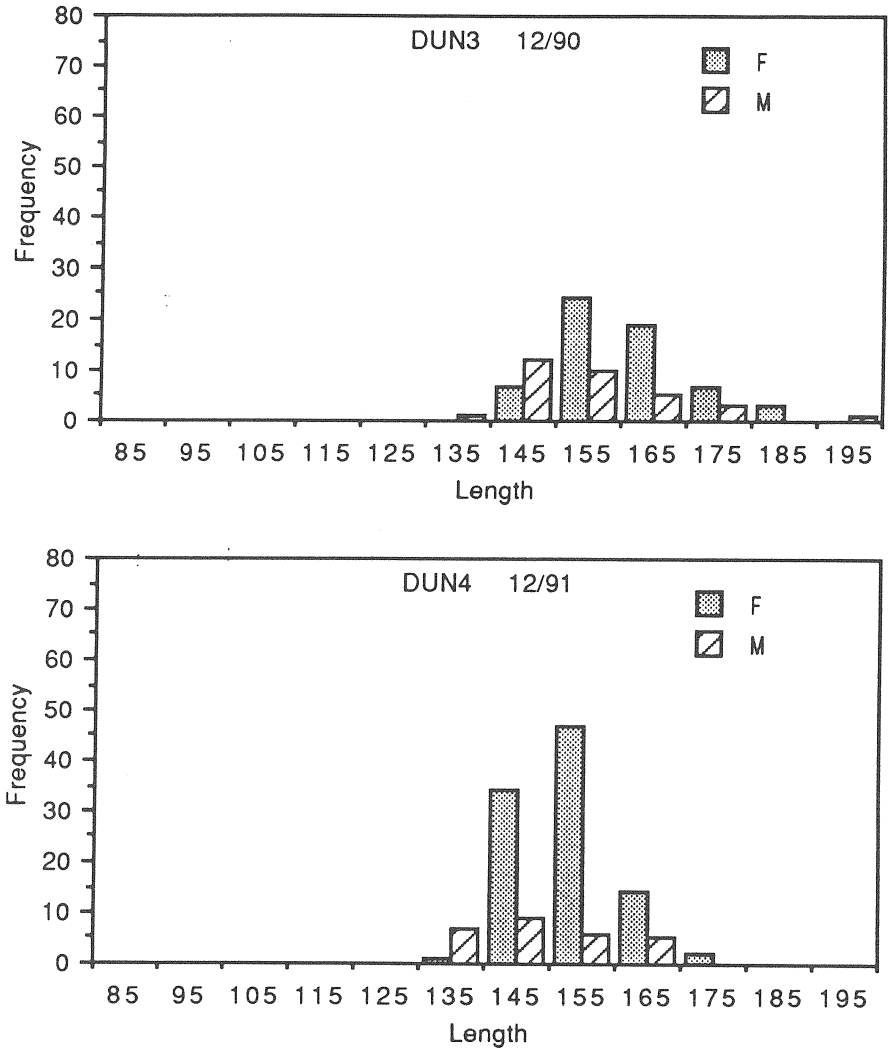


Appendix 4.4: Length frequency histograms for Torbay samples, WA

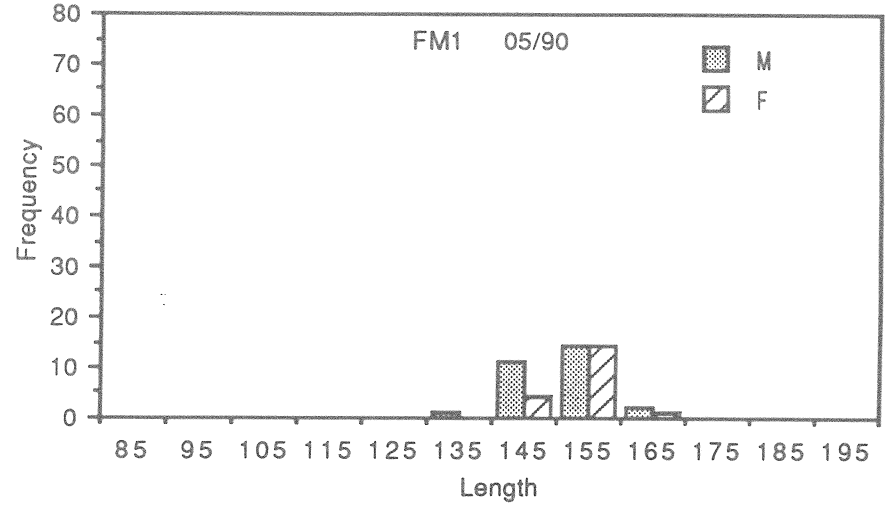


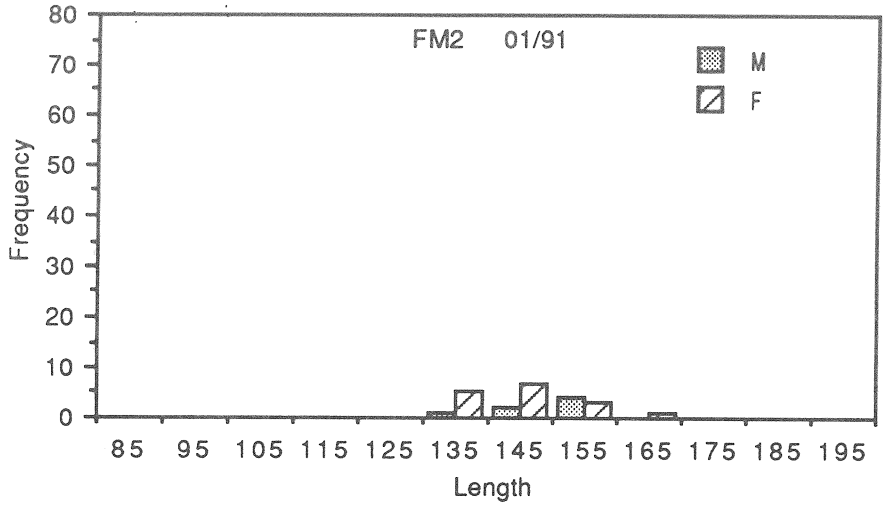


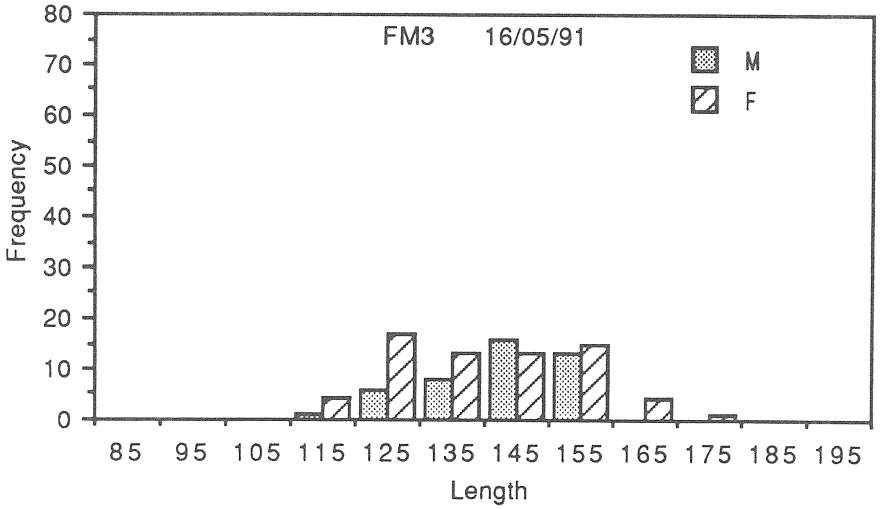
Appendix 4.4: Length frequency histograms for Dunsborough samples, WA



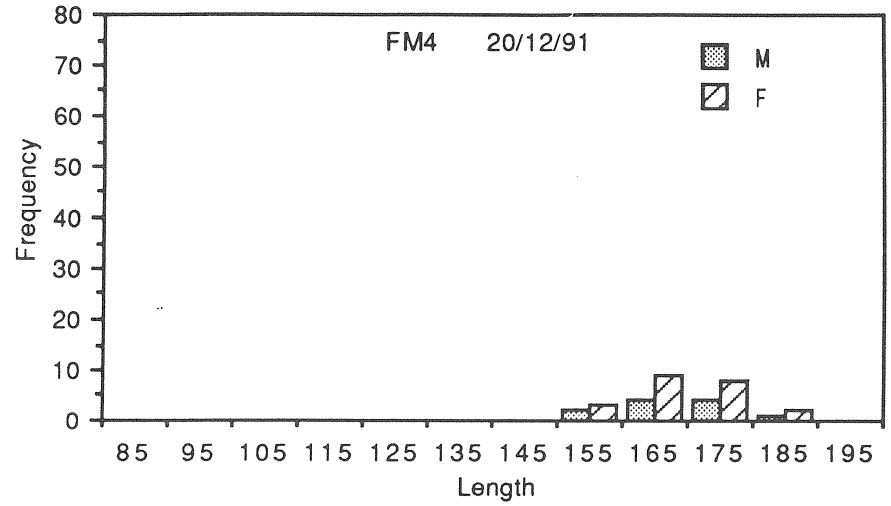
Appendix 4.5: Length frequency histograms for Dunsborough samples, WA



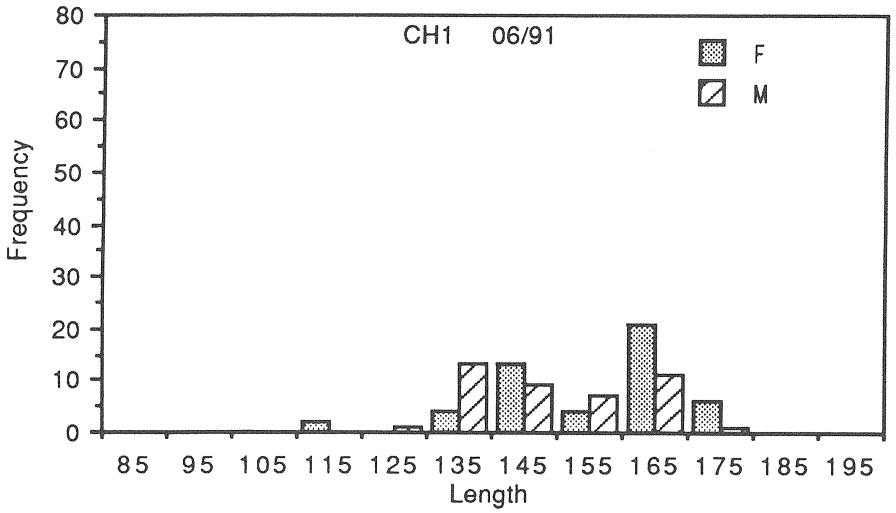




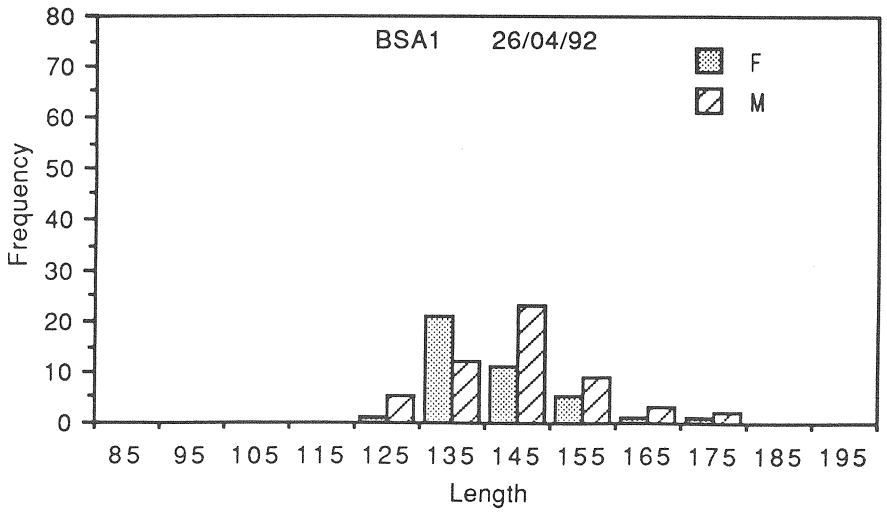
Appendix 4.6: Length frequency histograms for Fremantle samples, WA



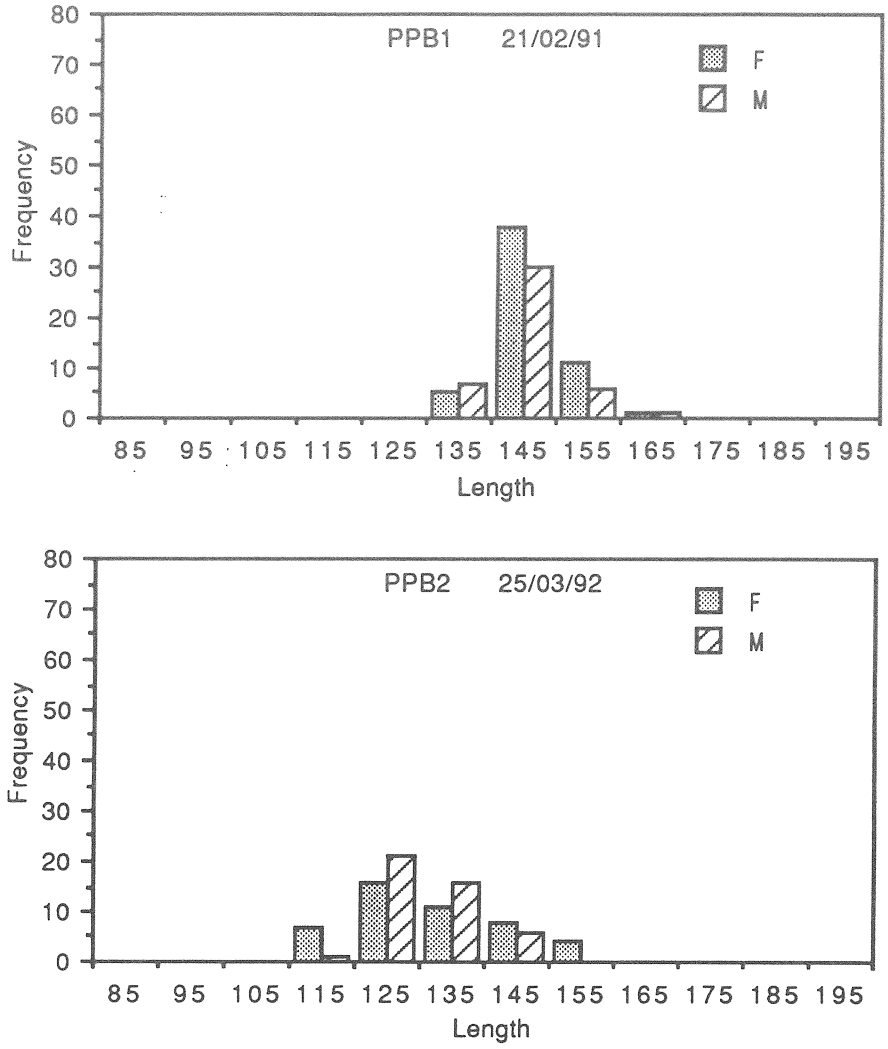
Appendix 4.6: Length frequency histograms for Fremantle samples, WA



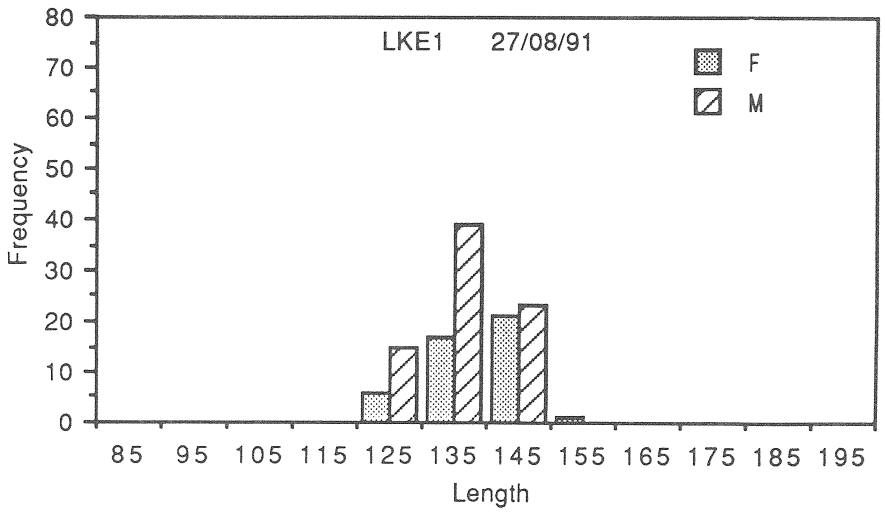
Appendix 4.7: Length frequency histograms for Cheynes Beach samples, WA



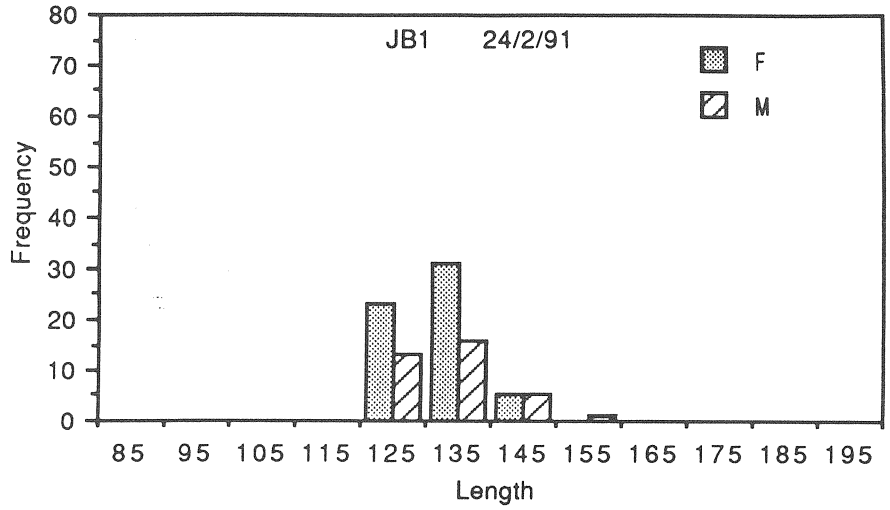
Appendix 4.8: Length frequency histograms for Boston Bay samples, SA

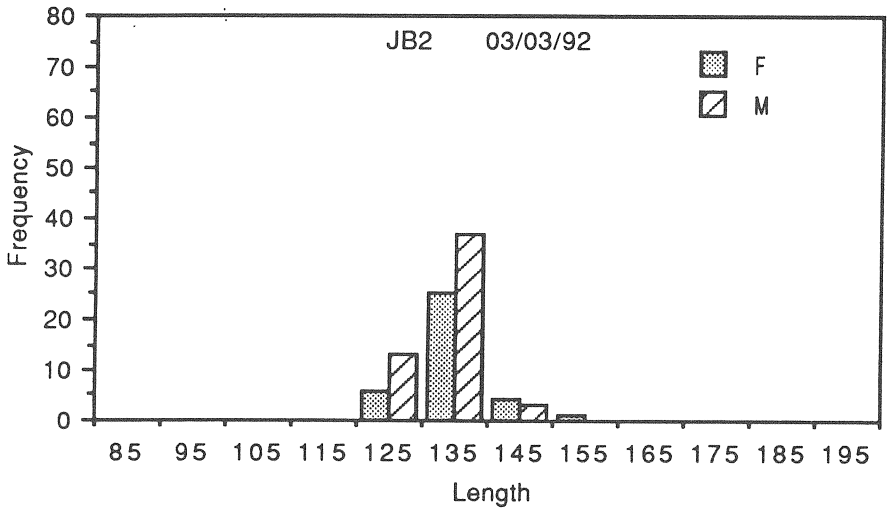


Appendix 4.9: Length frequency histograms for Port Phillip Bay samples, VIC



Appendix 4.9: Length frequency histograms for Lakes Entrance samples, VIC


JB1 24/2/91
F
M
Frequency
0
10
20
30
40
50
60
70
80
85 95 105 115 125 135 145 155 165 175 185 195
Length


JB2 03/03/92
F
M
Frequency
0
10
20
30
40
50
60
70
80
85 95 105 115 125 135 145 155 165 175 185 195
Length

APPENDIX 5.0: Rare Allele Distribution

SAMPLE	DATE	*AH**	*MPI**	*PEPB**		*PGM**	
		80	*110*	*110*	*95*	*110*	*85*
PPB1	21/02/91	.01	.02	.01	.04	.01	
PPB2	25/03/92	.02	.02	.01	.02	.05	
LKE1	27/08/91	-	.03	.01	.04	.03	
JB1	24/02/91	-	.01	.01	.01	.04	
JB2	03/03/92	.01	.0	.01	.04	.03	.01
SAF1	??/01/91	-	-	-	.05	.08	
BSA1	26/04/92	.01	.02	-	.02	.02	
BB1	28+9/04/90	-	.01	-	.03	.05	
BB2	26+7/09/90	.03	.02	-	.02	.03	
BB3	12+01/90+91	.02	.025	-	.05	.025	
BB4	05+06/91	.02	.02	.004	.01	.03	
DUN1	00/05/90	-	.02	-	.02	.02	
DUN2	22/11/90	.01	.02	-	.03	.02	
DUN3	20+17/12/90	.01	-	-	.03	.02	
DUN4	16+06/12/91	.02	.01	.01	.015	.035	
ESP1	10/05/90	.02	.06	.02	-	.01	
ESP2	14+01/11/90	.01	-	.01	.04	.04	
ESP3(jvnls)	10/05/91	-	-	-	-	.05	
ESP4	03/04/91	-	.09	.04	-	.04	
ESP5	03+05/06/91	.025	.015	-	.035	.02	
ESP6	16+19/01/91	.005	.015	.005	.025	.065	
FM1	11/05/90	-	-	.03	-	-	
FM2	16+31/01/91	-	-	-	-	.02	
FM3	16/05/91	.01	-	.01	.02	.05	
FM4	20/12/91	-	-	.02	-	.06	
KGS1	30/03/90	.01	.06	-	.05	-	
KGS2	07+10/05/90	-	.06	-	.02	.03	
KGS3	06/09/90	.01	.02	.01	.02	.03	
KGS4	05+12/01/91	-	.02	.01	-	.05	
KGS5	05/04/91	-	.02	.01	.01	.03	
KGS6	10+13+21/05/91	-	.015	.01	.02	.012	
KGS7	06/12/91	-	.02	.01	.03	.02	
TOR1	24/05/90	-	.01	-	.01	.01	
TOR2	26/08/90	.02	.01	.01	.04	.01	
TOR3	12+01/90+91	.01	-	.01	.06	.02	
TOR4	12/06/91	-	.03	.01	.02	.03	
CHB1	12/6/91	.03	.02	.01	.06	.04	

APPENDIX 6.0: Allele Frequencies

Appendix 6.1: Allele Pooling Rationale.

For *AAT*, EST-4,* MPI*, PEPB** and *PGM** the least common alleles were pooled together. At the *AH** locus a sixth allele (**115*) appeared on rare occasions between alleles **120* and **110*. Due to little separation between these alleles scoring was difficult (and there may be samples where allele **115* was not detected). Thus, allele **115* was scored as **110* but in case of miscoring, alleles **120* (**115*) and **110* were pooled together. As the frequencies of **110* and **100* were often similar, alleles **90* and **80* were pooled with allele **100* to maintain its integrity as a common allele.

Pooling of alleles was as follows:

	LOCUS					
Pooled designate	*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
**A*	*120*	*120*	*110*	*110*	*110*	*110*
	110	*110*	*90*	*90*	*90*	*95*
						90
**B*	*100*	*100*	*100*	*100*	*100*	*100*
		90				
		80				

Appendix 6.2: Pooled Allele Frequencies for all WA Samples. (see Appendix 1 for sample codes.) *B* is the common allele.

SAMPLE	*AAT**		*ACON**		*EST-4**		*MPI**		*PEP-B**		*PGM**	
CODE	*B*	(N)	*B*	(N)	*B*	(N)	*B*	(N)	*B*	(N)	*B*	(N)
DUN1	0.457	23	0.545	22	0.750	20	0.909	22	0.886	22	0.750	22
DUN2	0.470	83	0.519	80	0.850	70	0.927	82	0.823	82	0.747	83
DUN3	0.494	78	0.547	75	0.797	74	0.948	77	0.854	79	0.810	79
DUN4	0.646	127	0.552	125	0.891	124	0.937	126	0.850	127	0.791	127
FM1	0.563	32	0.554	37	0.681	36	0.941	34	0.813	40	0.868	38
FM2	0.704	27	0.479	24	0.889	27	0.946	28	0.828	29	0.870	27
FM3	0.626	107	0.587	109	0.694	93	0.914	105	0.887	111	0.790	107
FM4	0.500	30	0.484	32	0.889	27	0.950	30	0.818	33	0.828	32
TOR1	0.486	37	0.448	29	0.886	35	0.929	35	0.861	36	0.833	36
TOR2	0.475	100	0.567	97	0.812	101	0.955	101	0.848	102	0.755	102
TOR3	0.543	69	0.517	60	0.843	67	0.964	69	0.843	70	0.736	70
TOR4	0.520	51	0.519	52	0.638	47	0.962	52	0.877	53	0.745	53
KGS1	0.500	43	0.607	42	0.817	41	0.910	39	0.911	45	0.762	40
KGS2	0.609	55	0.574	54	0.806	54	0.886	57	0.843	51	0.793	58
KGS3	0.596	136	0.516	127	0.694	72	0.938	138	0.839	137	0.796	137
KGS4	0.552	58	0.420	56	0.683	52	0.907	54	0.862	58	0.737	57
KGS5	0.546	76	0.488	80	0.711	71	0.930	79	0.846	81	0.796	81
KGS6	0.627	106	0.614	105	0.821	95	0.938	105	0.854	106	0.710	105
KGS7	0.578	109	0.587	69	0.885	96	0.929	98	0.862	105	0.809	102
BB1	0.573	41	0.489	44	0.567	45	0.966	44	0.823	48	0.685	46
BB2	0.580	50	0.551	49	0.896	48	0.908	49	0.860	50	0.810	50
BB3	0.600	80	0.563	80	0.750	76	0.917	78	0.844	80	0.706	80
BB4	0.565	124	0.606	123	0.750	118	0.948	126	0.845	126	0.756	125
CHB1	0.527	93	0.602	93	0.759	81	0.930	93	0.826	95	0.689	95
ESP1	0.525	61	0.576	59	0.750	58	0.818	55	0.897	63	0.754	57
ESP2	0.534	58	0.617	47	0.791	55	0.911	56	0.836	58	0.763	57
ESP3	0.727	22	0.636	22	0.886	22	0.977	22	0.841	22	0.619	21
ESP4	0.614	22	0.458	24	0.841	22	0.804	23	0.840	25	0.820	25
ESP5	0.567	45	0.522	46	0.867	45	0.956	45	0.880	46	0.859	46
ESP6	0.541	74	0.582	67	0.924	66	0.957	70	0.818	74	0.767	75

Appendix 6.2 (cont): Pooled Allele Frequencies for non WA samples (before pooling).(see Appendix 1 for sample codes.)

SAMPLE	*AAT**		*ACON**		*EST-4**		*MPI**		*PEP-B**		*PGM**	
CODE	*B*	(N)	*B*	(N)	*B*	(N)	*B*	(N)	*B*	(N)	*B*	(N)
BSA1	0.600	95	0.521	95	0.932	96	0.932	96	0.818	96	0.760	96
PPB1	0.561	99	0.515	99	0.872	98	0.954	97	0.843	99	0.727	99
PPB2	0.593	91	0.522	90	0.892	88	0.918	92	0.806	93	0.766	94
LKE1	0.552	115	0.484	91	0.877	118	0.901	116	0.828	122	0.737	118
JB1	0.672	99	0.616	99	0.908	98	0.960	99	0.848	99	0.753	99
JB2	0.656	93	0.516	96	0.900	95	0.932	96	0.797	96	0.729	96
SAF	0.609	32	0.414	29	0.804	28	0.953	32	0.922	32	0.813	32

APPENDIX 7.0: Tests of Fit to Hardy Weinberg Equilibrium

Appendix 7.1: Chi-square test for deviation from Hardy-Weinberg equilibrium for each initial sample

Table 7.1.1: King George Sound, WA., 30 03 90 (KGS1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	17	10.624			
	A-B	9	21.753			
	B-B	17	10.624			
				15.131	1	.000
*AH**						
	A-A	8	6.361			
	A-B	17	20.277			
	B-B	17	15.361			
				1.126	1	.289
*EST-4**						
	A-A	3	1.296			
	A-B	9	12.407			
	B-B	29	27.296			
				3.281	1	.070
*MPI**						
	A-A	0	.273			
	A-B	7	6.455			
	B-B	32	32.273			
				.321	1	.571
*PEPB**						
	A-A	2	.315			
	A-B	4	7.371			
	B-B	39	37.315			
				10.647	1	.001
*PGM**						
	A-A	2	2.165			
	A-B	15	14.671			
	B-B	23	23.165			
				.021	1	.885

Table 7.1.1: King George Sound, WA., 00 05 90 (KGS2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	15	8.284			
	A-B	13	26.431			
	B-B	27	20.284			
				14.492	1	.000
*AH**						
	A-A	13	9.673			
	A-B	20	26.654			
	B-B	21	17.673			
				3.432	1	.064
*EST-4**						
	A-A	5	1.963			
	A-B	11	17.075			
	B-B	38	34.963			
				7.126	1	.008
*MPI**						
	A-A	2	.690			
	A-B	9	11.619			
	B-B	46	44.690			
				3.114	1	.078
*PEPB**						
	A-A	3	1.188			
	A-B	10	13.624			
	B-B	38	36.188			
				3.818	1	.051
*PGM**						
	A-A	2	2.400			
	A-B	20	19.200			
	B-B	36	36.400			
				.104	1	.747

The *AAT** and *EST-4*** loci were not in Hardy Weinberg equilibrium ($p<0.05$) but as *AH**, *MPI** and *PEPB** were close to being significantly out, this sample was considered to be heterogeneous.

Table 7.1.1: King George Sound, WA 00 09 90 (KGS3)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	33	22.122			
	A-B	44	65.756			
	B-B	59	48.122			
				15.007	1	.000
*AH**						
	A-A	36	29.656			
	A-B	51	63.688			
	B-B	40	33.656			
				5.080	1	.024
*EST-4**						
	A-A	11	6.615			
	A-B	22	30.769			
	B-B	39	34.615			
				5.961	1	.015
*MPI**						
	A-A	1	.495			
	A-B	15	16.011			
	B-B	122	121.495			
				.583	1	.445
*PEPB**						
	A-A	3	3.465			
	A-B	38	37.070			
	B-B	96	96.465			
				.088	1	.767
*PGM**						
	A-A	7	5.641			
	A-B	42	44.718			
	B-B	88	86.641			
				.514	1	.473

Three of the six loci from this sample were significantly out of Hardy Weinberg equilibrium, so it was considered to be heterogeneous.

Table 7.1.1: King George Sound, WA, 00 01 91 (KGS4)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	14	11.530			
	A-B	24	28.939			
	B-B	20	17.530			
				1.720	1	.190
*AH**						
	A-A	22	18.739			
	A-B	21	27.523			
	B-B	13	9.739			
				3.205	1	.073
*EST-4**						
	A-A	7	5.126			
	A-B	19	22.748			
	B-B	26	24.126			
				1.448	1	.229
*MPI**						
	A-A	0	.421			
	A-B	10	9.159			
	B-B	44	44.421			
				.502	1	.479
*PEPB**						
	A-A	2	1.043			
	A-B	12	13.913			
	B-B	44	43.043			
				1.161	1	.281
*PGM**						
	A-A	7	3.850			
	A-B	16	22.301			
	B-B	34	30.850			
				4.680	1	.031

*PGM** deviated significantly from Hardy Weinberg equilibrium ($p<0.05$) and *AH** was close so it was considered to be heterogeneous.

Table 7.1.1: King George Sound, WA. 05 04 91 (KGS5)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	23	15.536			
	A-B	23	37.927			
	B-B	30	22.536			
				11.932	1	.001
*AH**						
	A-A	26	20.887			
	A-B	30	40.226			
	B-B	24	18.887			
				5.236	1	.022
*EST-4**						
	A-A	8	5.816			
	A-B	25	29.369			
	B-B	38	35.816			
				1.604	1	.205
*MPI**						
	A-A	0	.350			
	A-B	11	10.299			
	B-B	68	68.350			
				.400	1	.527
*PEPB**						
	A-A	2	1.863			
	A-B	21	21.273			
	B-B	58	57.863			
				.014	1	.906
*PGM**						
	A-A	3	3.280			
	A-B	27	26.441			
	B-B	51	51.280			
				.037	1	.847

Table 7.1.1: King George Sound, WA. 00 05 91 (KGS6)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	22	14.602			
	A-B	35	49.796			
	B-B	49	41.602			
				9.460	1	.002
*AH**						
	A-A	18	15.502			
	A-B	45	49.995			
	B-B	42	39.502			
				1.059	1	.303
*EST-4**						
	A-A	4	2.968			
	A-B	26	28.063			
	B-B	65	63.968			
				.527	1	.468
*MPI**						
	A-A	3	.373			
	A-B	7	12.254			
	B-B	95	92.373			
				20.816	1	.000
*PEPB**						
	A-A	3	2.204			
	A-B	25	26.592			
	B-B	78	77.204			
				.391	1	.532
*PGM**						
	A-A	12	8.756			
	A-B	37	43.488			
	B-B	56	52.756			
				2.369	1	.124

Table7.1.1: King George Sound, WA. 06 12 91 (KGS7)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	26	19.290			
	A-B	40	53.419			
	B-B	43	36.290			
				6.945	1	.008
*AH**						
	A-A	16	11.650			
	A-B	25	33.701			
	B-B	28	23.650			
				4.671	1	.031
*EST-4**						
	A-A	0	1.209			
	A-B	22	19.581			
	B-B	74	75.209			
				1.528	1	.216
*MPI**						
	A-A	0	.467			
	A-B	14	13.067			
	B-B	84	84.467			
				.536	1	.464
*PEPB**						
	A-A	2	1.943			
	A-B	25	25.115			
	B-B	78	77.943			
				.002	1	.962
*PGM**						
	A-A	7	3.650			
	A-B	25	31.700			
	B-B	70	66.650			
				4.658	1	.031

As *AAT**, *AH** and *PGM** were significantly out of Hardy Weinberg equilibrium this sample was considered to be heterogeneous.

Table 7.1.2: Esperance, WA., 05 90 (ESP1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	26	13.661			
	A-B	6	30.678			
	B-B	29	16.661			
				40.134	1	.000
*AH**						
	A-A	17	10.470			
	A-B	16	29.060			
	B-B	26	19.470			
				12.132	1	.000
*EST-4**						
	A-A	6	3.530			
	A-B	17	21.939			
	B-B	35	32.530			
				3.027	1	.082
*MPI**						
	A-A	4	1.743			
	A-B	12	16.514			
	B-B	39	36.743			
				4.294	1	.038
*PEPB**						
	A-A	2	.624			
	A-B	9	11.752			
	B-B	52	50.624			
				3.716	1	.054
*PGM**						
	A-A	6	3.345			
	A-B	16	21.310			
	B-B	35	32.345			
				3.648	1	.056

Three loci were significantly out of Hardy Weinberg, and the other three loci were close, so it was considered to be a heterogeneous sample.

Table 7.1.2: Esperance, WA 11 90 (ESP2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	15	12.443			
	A-B	24	29.113			
	B-B	19	16.443			
				1.821	1	.177
*AH**						
	A-A	9	6.774			
	A-B	18	22.452			
	B-B	20	17.774			
				1.893	1	.169
*EST-4**						
	A-A	3	2.321			
	A-B	17	18.358			
	B-B	35	34.321			
				.312	1	.576
*MPI**						
	A-A	1	.405			
	A-B	8	9.189			
	B-B	47	46.405			
				1.034	1	.309
*PEPB**						
	A-A	0	1.487			
	A-B	19	16.026			
	B-B	39	40.487			
				2.093	1	.148
*PGM**						
	A-A	5	3.106			
	A-B	17	20.788			
	B-B	35	33.106			
				1.953	1	.162

Table 7.1.2: Esperance, WA. 10 05 91 (ESP3)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	3	1.535			
	A-B	6	8.930			
	B-B	13	11.535			
				2.546	1	.111
*AH**						
	A-A	4	2.791			
	A-B	8	10.419			
	B-B	10	8.791			
				1.252	1	.263
*EST-4**						
	A-A	1	.233			
	A-B	3	4.535			
	B-B	18	17.233			
				3.086	1	.079
*MPI**						
	A-A	0	.000			
	A-B	1	1.000			
	B-B	21	21.000			
				.000	1	1.000
*PEPB**						
	A-A	1	.488			
	A-B	5	6.023			
	B-B	16	15.488			
				.727	1	.394
*PGM**						
	A-A	3	2.927			
	A-B	10	10.146			
	B-B	8	7.927			
				.005	1	.946

Table 7.1.2: Esperance, WA. 03 04 91 (ESP4)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	6	3.163			
	A-B	5	10.674			
	B-B	11	8.163			
				6.548	1	.011
*AH**						
	A-A	8	6.915			
	A-B	10	12.170			
	B-B	6	4.915			
				.797	1	.372
*EST-4**						
	A-A	0	.488			
	A-B	7	6.023			
	B-B	15	15.488			
				.662	1	.416
*MPI**						
	A-A	1	.800			
	A-B	7	7.400			
	B-B	15	14.800			
				.074	1	.785
*PEPB**						
	A-A	0	.571			
	A-B	8	6.857			
	B-B	17	17.571			
				.780	1	.377
*PGM**						
	A-A	0	.735			
	A-B	9	7.531			
	B-B	16	16.735			
				1.054	1	.305

Table 7.1.2: Esperance, WA. 06 91 (ESP5)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	11	8.326			
	A-B	17	22.348			
	B-B	17	14.326			
				2.638	1	.104
*AH**						
	A-A	12	10.396			
	A-B	20	23.209			
	B-B	14	12.396			
				.899	1	.343
*EST-4**						
	A-A	2	.742			
	A-B	8	10.517			
	B-B	35	33.742			
				2.785	1	.095
*MPI**						
	A-A	0	.067			
	A-B	4	3.865			
	B-B	41	41.067			
				.072	1	.788
*PEPB**						
	A-A	2	.604			
	A-B	7	9.791			
	B-B	37	35.604			
				4.073	1	.044
*PGM**						
	A-A	1	.857			
	A-B	11	11.286			
	B-B	34	33.857			
				.032	1	.859

Table 7.1.2: Esperance, WA. 01 92 (ESP6)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	20	15.497			
	A-B	28	37.007			
	B-B	26	21.497			
				4.444	1	.035
*AH**						
	A-A	13	11.579			
	A-B	30	32.842			
	B-B	24	22.579			
				.510	1	.475
*EST-4**						
	A-A	0	.344			
	A-B	10	9.313			
	B-B	56	56.344			
				.396	1	.529
*MPI**						
	A-A	1	.108			
	A-B	4	5.784			
	B-B	65	64.108			
				7.937	1	.005
*PEPB**						
	A-A	2	2.388			
	A-B	23	22.224			
	B-B	49	49.388			
				.093	1	.760
*PGM**						
	A-A	3	3.993			
	A-B	29	27.013			
	B-B	43	43.993			
				.416	1	.519

Table 7.1.3: Bremer Bay, WA., 04 90 (BB1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	12	7.346			
	A-B	11	20.309			
	B-B	18	13.346			
				8.839	1	.003
*AH**						
	A-A	11	11.379			
	A-B	23	22.241			
	B-B	10	10.379			
				.052	1	.819
*EST-4**						
	A-A	10	8.326			
	A-B	19	22.348			
	B-B	16	14.326			
				1.034	1	.309
*MPI**						
	A-A	0	.034			
	A-B	3	2.931			
	B-B	41	41.034			
				.036	1	.849
*PEPB**						
	A-A	2	1.432			
	A-B	13	14.137			
	B-B	33	32.432			
				.327	1	.567
*PGM**						
	A-A	6	4.462			
	A-B	17	20.077			
	B-B	23	21.462			
				1.112	1	.292

Table 7.1.3: Bremer Bay, WA. 09 90 (BB2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	16	8.697			
	A-B	10	24.606			
	B-B	24	16.697			
				17.997	1	.000
*AH**						
	A-A	13	9.753			
	A-B	18	24.495			
	B-B	18	14.753			
				3.518	1	.061
*EST-4**						
	A-A	1	.474			
	A-B	8	9.053			
	B-B	39	38.474			
				.714	1	.398
*MPI**						
	A-A	1	.371			
	A-B	7	8.258			
	B-B	41	40.371			
				1.267	1	.260
*PEPB**						
	A-A	2	.919			
	A-B	10	12.162			
	B-B	38	36.919			
				1.687	1	.194
*PGM**						
	A-A	0	1.727			
	A-B	19	15.545			
	B-B	31	32.727			
				2.586	1	.108

Table 7.1.3: Bremer Bay, WA. Summer 90 (BB3)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	22	12.679			
	A-B	20	38.642			
	B-B	38	28.679			
				18.874	1	.000
*AH**						
	A-A	19	15.189			
	A-B	32	39.623			
	B-B	29	25.189			
				3.000	1	.083
*EST-4**						
	A-A	6	4.656			
	A-B	26	28.689			
	B-B	44	42.656			
				.683	1	.409
*MPI**						
	A-A	0	.503			
	A-B	13	11.994			
	B-B	65	65.503			
				.592	1	.442
*PEPB**						
	A-A	4	1.887			
	A-B	17	21.226			
	B-B	59	56.887			
				3.287	1	.070
*PGM**						
	A-A	10	6.799			
	A-B	27	33.403			
	B-B	43	39.799			
				2.992	1	.084

Table 7.1.3: Bremer Bay, WA. 05 91 (BB4)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	37	23.393			
	A-B	34	61.215			
	B-B	53	39.393			
				24.715	1	.000
*AH**						
	A-A	31	19.004			
	A-B	35	58.992			
	B-B	57	45.004			
				20.527	1	.000
*EST-4**						
	A-A	12	7.281			
	A-B	35	44.438			
	B-B	71	66.281			
				5.399	1	.020
*MPI**						
	A-A	1	.311			
	A-B	11	12.378			
	B-B	114	113.311			
				1.686	1	.194
*PEPB**						
	A-A	2	2.952			
	A-B	35	33.096			
	B-B	89	89.952			
				.427	1	.514
*PGM**						
	A-A	8	7.349			
	A-B	45	46.301			
	B-B	72	71.349			
				.100	1	.752

This sample was considered to be heterogeneous as three loci were significantly out of Hardy Weinberg equilibrium ($p<0.05$).

Table 7.1.4: Torbay, WA 24 05 90 (TOR1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	11	9.630			
	A-B	16	18.740			
	B-B	10	8.630			
				.813	1	.367
*AH**						
	A-A	7	8.702			
	A-B	18	14.596			
	B-B	4	5.702			
				1.634	1	.201
*EST-4**						
	A-A	0	.406			
	A-B	8	7.188			
	B-B	27	27.406			
				.503	1	.478
*MPI**						
	A-A	1	.145			
	A-B	3	4.710			
	B-B	31	30.145			
				5.690	1	.017
*PEPB**						
	A-A	2	.634			
	A-B	6	8.732			
	B-B	28	26.634			
				3.870	1	.049
*PGM**						
	A-A	0	.930			
	A-B	12	10.141			
	B-B	24	24.930			
				1.305	1	.253

Table 7.1.4: Torbay, WA., 09 90 (TOR2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	39	27.437			
	A-B	27	50.126			
	B-B	34	22.437			
				21.501	1	.000
*AH**						
	A-A	19	18.062			
	A-B	46	47.876			
	B-B	32	31.062			
				.150	1	.698
*EST-4**						
	A-A	5	3.498			
	A-B	28	31.005			
	B-B	68	66.498			
				.971	1	.325
*MPI**						
	A-A	1	.179			
	A-B	7	8.642			
	B-B	93	92.179			
				4.082	1	.043
*PEPB**						
	A-A	4	2.291			
	A-B	23	26.419			
	B-B	75	73.291			
				1.758	1	.185
*PGM**						
	A-A	2	6.034			
	A-B	46	37.931			
	B-B	54	58.034			
				4.694	1	.030

Table 7.1.4: Torbay, WA. Summer 90 (TOR3)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	20	14.255			
	A-B	23	34.489			
	B-B	26	20.255			
				7.771	1	.005
*AH**						
	A-A	15	13.891			
	A-B	28	30.218			
	B-B	17	15.891			
				.329	1	.566
*EST-4**						
	A-A	2	1.579			
	A-B	17	17.842			
	B-B	48	47.579			
				.156	1	.693
*MPI**						
	A-A	0	.073			
	A-B	5	4.854			
	B-B	64	64.073			
				.077	1	.781
*PEPB**						
	A-A	2	1.662			
	A-B	18	18.676			
	B-B	50	49.662			
				.096	1	.757
*PGM**						
	A-A	5	4.791			
	A-B	27	27.417			
	B-B	38	37.791			
				.017	1	.898

Table 7.1.4: Torbay, WA. 12 06 91 (TOR4)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	18	11.644			
	A-B	13	25.713			
	B-B	20	13.644			
				12.717	1	.000
*AH**						
	A-A	17	11.893			
	A-B	16	26.214			
	B-B	19	13.893			
				8.049	1	.005
*EST-4**						
	A-A	7	6.032			
	A-B	20	21.935			
	B-B	20	19.032			
				.375	1	.540
*MPI**						
	A-A	0	.058			
	A-B	4	3.883			
	B-B	48	48.058			
				.062	1	.804
*PEPB**						
	A-A	0	.743			
	A-B	13	11.514			
	B-B	40	40.743			
				.948	1	.330
*PGM**						
	A-A	4	3.343			
	A-B	19	20.314			
	B-B	30	29.343			
				.229	1	.632

Table 7.1.5: Dunsborough, WA. 05 90 (DUN1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	10	6.667			
	A-B	5	11.667			
	B-B	8	4.667			
				7.857	1	.005
*AH**						
	A-A	6	4.419			
	A-B	8	11.163			
	B-B	8	6.419			
				1.852	1	.174
*EST-4**						
	A-A	2	1.154			
	A-B	6	7.692			
	B-B	12	11.154			
				1.057	1	.304
*MPI**						
	A-A	0	.140			
	A-B	4	3.721			
	B-B	18	18.140			
				.162	1	.688
*PEPB**						
	A-A	1	.233			
	A-B	3	4.535			
	B-B	18	17.233			
				3.086	1	.079
*PGM**						
	A-A	0	1.279			
	A-B	11	8.442			
	B-B	11	12.279			
				2.188	1	.139

Table 7.1.5: Dunsborough, WA. 22 11 90 (DUN2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	30	23.200			
	A-B	28	41.600			
	B-B	25	18.200			
				8.980	1	.003
*AH**						
	A-A	18	18.403			
	A-B	41	40.195			
	B-B	21	21.403			
				.032	1	.857
*EST-4**						
	A-A	2	1.511			
	A-B	17	17.978			
	B-B	51	50.511			
				.216	1	.642
*MPI**						
	A-A	0	.405			
	A-B	12	11.190			
	B-B	70	70.405			
				.466	1	.495
*PEPB**						
	A-A	3	2.491			
	A-B	23	24.018			
	B-B	56	55.491			
				.152	1	.697
*PGM**						
	A-A	2	5.218			
	A-B	38	31.564			
	B-B	43	46.218			
				3.521	1	.061

Table 7.1.5: Dunsborough, WA. 12 91 (DUN3)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	24	19.877			
	A-B	31	39.245			
	B-B	23	18.877			
				3.488	1	.062
*AH**						
	A-A	16	15.289			
	A-B	36	37.423			
	B-B	23	22.289			
				.110	1	.740
*EST-4**						
	A-A	4	2.959			
	A-B	22	24.082			
	B-B	48	46.959			
				.569	1	.451
*MPI**						
	A-A	0	.183			
	A-B	8	7.634			
	B-B	69	69.183			
				.201	1	.654
*PEPB**						
	A-A	3	1.611			
	A-B	17	19.777			
	B-B	59	57.611			
				1.620	1	.203
*PGM**						
	A-A	3	2.771			
	A-B	24	24.459			
	B-B	52	51.771			
				.029	1	.866

Table 7.1.5: Dunsborough, WA. 12 91 (DUN4)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	22	15.830			
	A-B	46	58.340			
	B-B	59	52.830			
				5.736	1	.017
*AH**						
	A-A	30	24.964			
	A-B	52	62.072			
	B-B	43	37.964			
				3.318	1	.069
*EST-4**						
	A-A	3	1.421			
	A-B	21	24.158			
	B-B	100	98.421			
				2.193	1	.139
*MPI**						
	A-A	0	.478			
	A-B	16	15.044			
	B-B	110	110.478			
				.541	1	.462
*PEPB**						
	A-A	2	2.779			
	A-B	34	32.443			
	B-B	91	91.779			
				.300	1	.584
*PGM**						
	A-A	6	5.447			
	A-B	41	42.107			
	B-B	80	79.447			
				.089	1	.765

Table 7.1.6: Fremantle, WA. 11 05 90 (FM1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	9	6.000			
	A-B	10	16.000			
	B-B	13	10.000			
				4.650	1	.031
*AH**						
	A-A	10	7.233			
	A-B	13	18.534			
	B-B	14	11.233			
				3.393	1	.065
*EST-4**						
	A-A	6	3.563			
	A-B	11	15.873			
	B-B	19	16.563			
				3.521	1	.061
*MPI**						
	A-A	0	.090			
	A-B	4	3.821			
	B-B	30	30.090			
				.098	1	.754
*PEPB**						
	A-A	2	1.329			
	A-B	11	12.342			
	B-B	27	26.329			
				.502	1	.479
*PGM**						
	A-A	1	.600			
	A-B	8	8.800			
	B-B	29	28.600			
				.345	1	.557

Table 7.1.6: Fremantle, WA. 01 91 (FM2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	5	2.264			
	A-B	6	11.472			
	B-B	16	13.264			
				6.480	1	.011
*AH**						
	A-A	6	6.383			
	A-B	13	12.234			
	B-B	5	5.383			
				.098	1	.754
*EST-4**						
	A-A	0	.283			
	A-B	6	5.434			
	B-B	21	21.283			
				.346	1	.557
*MPI**						
	A-A	1	.055			
	A-B	1	2.891			
	B-B	26	25.055			
				17.660	1	.000
*PEPB**						
	A-A	2	.789			
	A-B	6	8.421			
	B-B	21	19.789			
				2.626	1	.105
*PGM**						
	A-A	0	.396			
	A-B	7	6.208			
	B-B	20	20.396			
				.505	1	.477

Table 7.1.6: Fremantle, WA. 16 05 91 (FM3)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	22	14.836			
	A-B	36	50.329			
	B-B	49	41.836			
				8.766	1	.003
*AH**						
	A-A	23	18.456			
	A-B	44	53.088			
	B-B	42	37.456			
				3.225	1	.073
*EST-4**						
	A-A	12	8.627			
	A-B	33	39.746			
	B-B	48	44.627			
				2.719	1	.099
*MPI**						
	A-A	4	.732			
	A-B	10	16.536			
	B-B	91	87.732			
				17.293	1	.000
*PEPB**						
	A-A	2	1.357			
	A-B	21	22.285			
	B-B	88	87.357			
				.383	1	.536
*PGM**						
	A-A	6	4.648			
	A-B	33	35.704			
	B-B	68	66.648			
				.626	1	.429

Table 7.1.6: Fremantle, WA 20 12 91 (FM4)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	12	7.373			
	A-B	6	15.254			
	B-B	12	7.373			
				11.422	1	.001
*AH**						
	A-A	11	8.381			
	A-B	11	16.238			
	B-B	10	7.381			
				3.437	1	.064
*EST-4**						
	A-A	0	.283			
	A-B	6	5.434			
	B-B	21	21.283			
				.346	1	.557
*MPI**						
	A-A	0	.051			
	A-B	3	2.898			
	B-B	27	27.051			
				.055	1	.815
*PEPB**						
	A-A	1	1.015			
	A-B	10	9.969			
	B-B	22	22.015			
				.000	1	.985
*PGM**						
	A-A	1	.873			
	A-B	9	9.254			
	B-B	22	21.873			
				.026	1	.871

Table 7.1.7: Cheynes Beach, WA 13 6 91 (CHB1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	31	20.692			
	A-B	26	46.616			
	B-B	36	25.692			
				18.389	1	.000
*AH**						
	A-A	23	14.600			
	A-B	28	44.800			
	B-B	42	33.600			
				13.233	1	.000
*EST-4**						
	A-A	7	4.602			
	A-B	25	29.795			
	B-B	49	46.602			
				2.144	1	.143
*MPI**						
	A-A	1	.422			
	A-B	11	12.157			
	B-B	81	80.422			
				.908	1	.341
*PEPB**						
	A-A	3	2.794			
	A-B	27	27.413			
	B-B	65	64.794			
				.022	1	.882
*PGM**						
	A-A	9	9.053			
	A-B	41	40.894			
	B-B	45	45.053			
				.001	1	.980

Table 7.1.8: Boston Bay, South Australia, 26 04 92 (BSA1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	21	15.079			
	A-B	34	45.841			
	B-B	40	34.079			
				6.412	1	.011
*AH**						
	A-A	26	21.667			
	A-B	39	47.667			
	B-B	30	25.667			
				3.174	1	.075
*EST-4**						
	A-A	1	.408			
	A-B	11	12.183			
	B-B	84	83.408			
				.976	1	.323
*MPI**						
	A-A	1	.408			
	A-B	11	12.183			
	B-B	84	83.408			
				.976	1	.323
*PEPB**						
	A-A	0	3.115			
	A-B	35	28.770			
	B-B	61	64.115			
				4.616	1	.032
*PGM**						
	A-A	5	5.419			
	A-B	36	35.162			
	B-B	55	55.419			
				.055	1	.814

The excess of heterozygotes at the *PEPB** locus was possibly a chance occurrence.

Table 7.1.9: Port Phillip Bay, Vic. 21 02 91 (PPB1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	25	18.990			
	A-B	37	49.020			
	B-B	37	30.990			
				6.015	1	.014
*AH**						
	A-A	28	23.147			
	A-B	40	49.706			
	B-B	31	26.147			
				3.813	1	.051
*EST-4**						
	A-A	3	1.538			
	A-B	19	21.923			
	B-B	76	74.538			
				1.807	1	.179
*MPI**						
	A-A	0	.187			
	A-B	9	8.627			
	B-B	88	88.187			
				.203	1	.652
*PEPB**						
	A-A	5	2.360			
	A-B	21	26.279			
	B-B	73	70.360			
				4.111	1	.043
*PGM**						
	A-A	8	7.264			
	A-B	38	39.472			
	B-B	53	52.264			
				.140	1	.708

Table 7.1.9: Port Phillip Bay, VIC, 25 03 92 (PPB2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	23	14.923			
	A-B	28	44.155			
	B-B	40	31.923			
				12.326	1	.000
*AH**						
	A-A	20	20.419			
	A-B	46	45.162			
	B-B	24	24.419			
				.031	1	.859
*EST-4**						
	A-A	2	.977			
	A-B	15	17.046			
	B-B	71	69.977			
				1.331	1	.249
*MPI**						
	A-A	2	.574			
	A-B	11	13.852			
	B-B	79	77.574			
				4.159	1	.041
*PEPB**						
	A-A	5	3.405			
	A-B	26	29.189			
	B-B	62	60.405			
				1.137	1	.286
*PGM**						
	A-A	3	5.059			
	A-B	38	33.882			
	B-B	53	55.059			
				1.415	1	.234

Table 7.1.9: Lakes Entrance, Victoria, 27 08 91 (LKE1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	32	22.939			
	A-B	39	57.122			
	B-B	44	34.939			
				11.679	1	.001
*AH**						
	A-A	27	24.149			
	A-B	40	45.702			
	B-B	24	21.149			
				1.432	1	.231
*EST-4**						
	A-A	3	1.728			
	A-B	23	25.545			
	B-B	92	90.728			
				1.208	1	.272
*MPI**						
	A-A	2	1.095			
	A-B	19	20.810			
	B-B	95	94.095			
				.913	1	.339
*PEPB**						
	A-A	3	3.543			
	A-B	36	34.914			
	B-B	83	83.543			
				.121	1	.728
*PGM**						
	A-A	14	8.047			
	A-B	34	45.906			
	B-B	70	64.047			
				8.046	1	.005

The significant deviation from Hardy Weinberg equilibrium at the *PGM** locus ($p<0.05$) may indicate significant heterogeneity due to mixed size classes in this sample.

Table 7.1.10: Jervis Bay, NSW, 24 02 91 (JB1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	18	10.558			
	A-B	29	43.883			
	B-B	52	44.558			
				11.535	1	.001
*AH**						
	A-A	17	14.467			
	A-B	42	47.066			
	B-B	40	37.467			
				1.160	1	.281
*EST-4**						
	A-A	2	.785			
	A-B	14	16.431			
	B-B	82	80.785			
				2.261	1	.133
*MPI**						
	A-A	0	.142			
	A-B	8	7.716			
	B-B	91	91.142			
				.153	1	.696
*PEPB**						
	A-A	4	2.208			
	A-B	22	25.584			
	B-B	73	71.208			
				2.001	1	.157
*PGM**						
	A-A	4	5.970			
	A-B	41	37.061			
	B-B	54	55.970			
				1.138	1	.286

Table 7.1.10: Jervis Bay, NSW, 03 03 92 (JB2)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	16	10.897			
	A-B	32	42.205			
	B-B	45	39.897			
				5.510	1	.019
*AH**						
	A-A	30	22.398			
	A-B	33	48.204			
	B-B	33	25.398			
				9.651	1	.002
*EST-4**						
	A-A	3	.905			
	A-B	13	17.190			
	B-B	79	76.905			
				5.931	1	.015
*MPI**						
	A-A	2	.408			
	A-B	9	12.183			
	B-B	85	83.408			
				7.065	1	.008
*PEPB**						
	A-A	2	3.880			
	A-B	35	31.241			
	B-B	59	60.880			
				1.421	1	.233
*PGM**						
	A-A	9	6.942			
	A-B	34	38.115			
	B-B	53	50.942			
				1.137	1	.286

This sample was considered to be heterogeneous because four loci deviated significantly from Hardy Weinberg equilibrium ($p<0.05$) due to heterozygote deficits.

Table 7.1.11: South Africa, 00 01 91 (SAF1)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	8	4.762			
	A-B	9	15.476			
	B-B	15	11.762			
				5.803	1	.016
*AH**						
	A-A	9	9.842			
	A-B	16	14.316			
	B-B	4	4.842			
				.417	1	.519
*EST-4**						
	A-A	1	1.000			
	A-B	9	9.000			
	B-B	18	18.000			
				.000	1	1.000
*MPI**						
	A-A	1	.048			
	A-B	1	2.905			
	B-B	30	29.048			
				20.328	1	.000
*PEPB**						
	A-A	0	.159			
	A-B	5	4.683			
	B-B	27	27.159			
				.181	1	.670
*PGM**						
	A-A	3	1.048			
	A-B	6	9.905			
	B-B	23	21.048			
				5.359	1	.021

This sample may not be homogeneous as the *PGM** locus also was significantly out of Hardy Weinberg equilibrium.

Appendix 7.2: Chi-square test for deviation from Hardy Weinberg equilibrium for male and females only.

7.2.1: PPB2 FEMALES (PP2F)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	11	6.806			
	A-B	13	21.389			
	B-B	21	16.806			
				6.922	1	.009
*AH*1*						
	A-A	10	9.773			
	A-B	21	21.453			
	B-B	12	11.773			
				.019	1	.890
*EST-4**						
	A-A	1	.857			
	A-B	10	10.286			
	B-B	31	30.857			
				.032	1	.857
*MPI**						
	A-A	0	.091			
	A-B	4	3.818			
	B-B	40	40.091			
				.1	001	.752
*PEPB**						
	A-A	2	1.642			
	A-B	13	13.716			
	B-B	29	28.642			
				.120	1	.729
*PGM**						
	A-A	1	2.006			
	A-B	17	14.989			
	B-B	27	28.006			
				.810	1	.368

7.2.1: PPB2 MALES (PP2M)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	12	8.843			
	A-B	15	21.314			
	B-B	16	12.843			
				3.773	1	.052
*AH*1*						
	A-A	10	10.756			
	A-B	24	22.489			
	B-B	11	11.756			
				.203	1	.652
*EST-4**						
	A-A	1	.285			
	A-B	5	6.430			
	B-B	37	36.285			
				2.127	1	.145
*MPI**						
	A-A	2	.556			
	A-B	6	8.889			
	B-B	37	35.556			
				4.753	1	.029
*PEPB**						
	A-A	3	1.962			
	A-B	13	15.076			
	B-B	30	28.962			
				.872	1	.350
*PGM**						
	A-A	2	2.875			
	A-B	19	17.250			
	B-B	25	25.875			
				.473	1	.491

Appendix 7.2.3: Contingency chi-square analysis at all loci between PPB males and females.

Locus	No. of alleles	Chi-square	D.F.	P
*AAT**-1	2	.753	1	.38548
*AH*1*	2	.026	1	.87196
*EST-4**	2	1.617	1	.20351
*MPI**	2	2.647	1	.10376
*PEPB**	2	.050	1	.82303
*PGM**	2	.388	1	.53357
(Totals)		5.481	6	.48381

There were no significant differences ($p<0.05$) between males and females at any loci.

Appendix 7.3: Tests for conformity to Hardy Weinberg equilibrium of pooled samples from temporal variation analyses.

Table 7.3.1: KGS2+5+6 WINTER SPAWNERS (KGS winter spawners)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	60	38.482			
	A-B	71	114.036			
	B-B	106	84.482			
				33.754	1	.000
*AH**						
	A-A	57	45.691			
	A-B	95	117.617			
	B-B	87	75.691			
				8.838	1	.003
*EST-4**						
	A-A	17	10.473			
	A-B	62	75.055			
	B-B	141	134.473			
				6.656	1	.010
*MPI**						
	A-A	5	1.420			
	A-B	27	34.160			
	B-B	209	205.420			
				10.587	1	.001
*PEPB**						
	A-A	8	5.445			
	A-B	56	61.109			
	B-B	174	171.445			
				1.664	1	.197
*PGM**						
	A-A	17	14.266			
	A-B	84	89.467			
	B-B	143	140.266			
			.911	1	.340	

Table 7.3.1: KGS1+3 AUT/SPR non spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	50	32.694			
	A-B	53	87.612			
	B-B	76	58.694			
				27.937	1	.000
*AH**						
	A-A	44	36.000			
	A-B	68	84.000			
	B-B	57	49.000			
				6.132	1	.013
*EST-4**						
	A-A	14	7.701			
	A-B	31	43.597			
	B-B	68	61.701			
				9.434	1	.002
*MPI**						
	A-A	1	.814			
	A-B	22	22.373			
	B-B	154	153.814			
				.049	1	.825
*PEPB**						
	A-A	5	3.714			
	A-B	42	44.571			
	B-B	135	133.714			
				.606	1	.436
*PGM**						
	A-A	9	7.945			
	A-B	57	59.110			
	B-B	111	109.945			
				.226	1	.635

Table 7.3.2: ESP4+5 Winter spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	17	11.701			
	A-B	22	32.597			
	B-B	28	22.701			
				7.081	1	.008
*AH**						
	A-A	20	17.500			
	A-B	30	35.000			
	B-B	20	17.500			
				1.429	1	.232
*EST-4**						
	A-A	2	1.347			
	A-B	15	16.306			
	B-B	50	49.347			
				.430	1	.512
*MPI**						
	A-A	1	.621			
	A-B	11	11.757			
	B-B	56	55.621			
				.282	1	.595
*PEPB**						
	A-A	2	1.271			
	A-B	15	16.458			
	B-B	54	53.271			
				.557	1	.455
*PGM**						
	A-A	1	1.704			
	A-B	20	18.592			
	B-B	50	50.704			
				.407	1	.523

Table 7.3.2: ESP 6+2 summer non-spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	35	28.189			
	A-B	52	65.621			
	B-B	45	38.189			
				5.687	1	.017
*AH**						
	A-A	22	18.561			
	A-B	48	54.877			
	B-B	44	40.561			
				1.790	1	.181
*EST-4**						
	A-A	3	2.250			
	A-B	27	28.500			
	B-B	91	90.250			
				.335	1	.563
*MPI**						
	A-A	2	.508			
	A-B	12	14.984			
	B-B	112	110.508			
				4.997	1	.025
*PEPB**						
	A-A	2	4.008			
	A-B	42	37.985			
	B-B	88	90.008			
				1.475	1	.225
*PGM**						
	A-A	8	7.280			
	A-B	46	47.439			
	B-B	78	77.280			
				.122	1	.727

Table 7.3.3: BB1+3 WA Winter and summer

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	34	20.250			
	A-B	31	58.500			
	B-B	56	42.250			
				26.739	1	.000
*AH**						
	A-A	30	26.663			
	A-B	55	61.673			
	B-B	39	35.663			
				1.452	1	.228
*EST-4**						
	A-A	16	12.250			
	A-B	45	52.500			
	B-B	60	56.250			
				2.469	1	.116
*MPI**						
	A-A	0	.525			
	A-B	16	14.951			
	B-B	106	106.525			
				.601	1	.438
*PEPB**						
	A-A	6	3.445			
	A-B	30	35.109			
	B-B	92	89.445			
				2.711	1	.100
*PGM**						
	A-A	16	11.460			
	A-B	44	53.079			
	B-B	66	61.460			
				3.687	1	.055

Table 7.3.4: TOR1+4 winter spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	29	21.503			
	A-B	29	43.994			
	B-B	30	22.503			
				10.222	1	.001
*AH**						
	A-A	24	20.753			
	A-B	34	40.494			
	B-B	23	19.753			
				2.083	1	.149
*EST-4**						
	A-A	7	5.378			
	A-B	28	31.244			
	B-B	47	45.378			
				.884	1	.347
*MPI**						
	A-A	1	.233			
	A-B	7	8.534			
	B-B	79	78.233			
				2.812	1	.094
*PEPB**						
	A-A	2	1.486			
	A-B	19	20.028			
	B-B	68	67.486			
				.235	1	.628
*PGM**						
	A-A	4	4.272			
	A-B	31	30.455			
	B-B	54	54.272			
				.028	1	.866

Table 7.3.4: TOR1+4+3 winter and summer spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	49	35.828			
	A-B	52	78.344			
	B-B	56	42.828			
				17.752	1	.000
*AH**						
	A-A	39	34.752			
	A-B	62	70.496			
	B-B	40	35.752			
				2.048	1	.152
*EST-4**						
	A-A	9	6.659			
	A-B	45	49.681			
	B-B	95	92.659			
				1.323	1	.250
*MPI**						
	A-A	1	.314			
	A-B	12	13.372			
	B-B	143	142.314			
				1.642	1	.200
*PEPB**						
	A-A	4	3.184			
	A-B	37	38.632			
	B-B	118	117.184			
				.284	1	.594
*PGM**						
	A-A	9	9.082			
	A-B	58	57.836			
	B-B	92	92.082			
				.001	1	.972

Table 7.3.5: DUN2+3 Summer spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	54	43.306			
	A-B	59	80.388			
	B-B	48	37.306			
				11.397	1	.001
*AH**						
	A-A	34	33.911			
	A-B	77	77.177			
	B-B	44	43.911			
				.001	1	.977
*EST-4**						
	A-A	6	4.516			
	A-B	39	41.969			
	B-B	99	97.516			
				.721	1	.396
*MPI**						
	A-A	0	.629			
	A-B	20	18.742			
	B-B	139	139.629			
				.716	1	.397
*PEPB**						
	A-A	6	4.199			
	A-B	40	43.602			
	B-B	115	113.199			
				1.099	1	.294
*PGM**						
	A-A	5	8.000			
	A-B	62	56.000			
	B-B	95	98.000			
				1.860	1	.173

Table 7.3.5: DUN2+3+1 summer spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	64	50.087			
	A-B	64	91.826			
	B-B	56	42.087			
				16.896	1	.000
*AH**						
	A-A	40	38.453			
	A-B	85	88.093			
	B-B	52	50.453			
				.218	1	.640
*EST-4**						
	A-A	8	5.672			
	A-B	45	49.655			
	B-B	111	108.672			
				1.442	1	.230
*MPI**						
	A-A	0	.796			
	A-B	24	22.409			
	B-B	157	157.796			
				.913	1	.339
*PEPB**						
	A-A	7	4.439			
	A-B	43	48.123			
	B-B	133	130.439			
				2.074	1	.150
*PGM**						
	A-A	5	9.360			
	A-B	73	64.280			
	B-B	106	110.360			
				3.386	1	.066

Table 7.3.6: FM1+3 winter non-spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	31	20.978			
	A-B	46	66.043			
	B-B	62	51.978			
				12.802	1	.000
*AH**						
	A-A	33	25.906			
	A-B	57	71.188			
	B-B	56	48.906			
				5.800	1	.016
*EST-4**						
	A-A	18	12.403			
	A-B	44	55.194			
	B-B	67	61.403			
				5.306	1	.021
*MPI**						
	A-A	4	.871			
	A-B	14	20.259			
	B-B	121	117.871			
				13.267	1	.000
*PEPB**						
	A-A	4	2.649			
	A-B	32	34.702			
	B-B	115	113.649			
				.915	1	.339
*PGM**						
	A-A	7	5.216			
	A-B	41	44.569			
	B-B	97	95.216			
				.930	1	.335

Table 7.3.6: FM2+3 WA

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	27	17.194			
	A-B	42	61.612			
	B-B	65	55.194			
				13.577	1	.000
*AH**						
	A-A	29	24.859			
	A-B	57	65.282			
	B-B	47	42.859			
				2.141	1	.143
*EST-4**						
	A-A	12	8.269			
	A-B	39	46.463			
	B-B	69	65.269			
				3.096	1	.079
*MPI**						
	A-A	5	.829			
	A-B	11	19.342			
	B-B	117	112.829			
				24.740	1	.000
*PEPB**						
	A-A	4	2.188			
	A-B	27	30.625			
	B-B	109	107.188			
				1.962	1	.161
*PGM**						
	A-A	6	5.045			
	A-B	40	41.910			
	B-B	88	87.045			
				.278	1	.598

Table 7.3.7: PPB1+2 non spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	48	34.107			
	A-B	65	92.787			
	B-B	77	63.107			
				17.040	1	.000
*AH**						
	A-A	48	43.815			
	A-B	86	94.370			
	B-B	55	50.815			
				1.487	1	.223
*EST-4**						
	A-A	5	2.602			
	A-B	34	38.796			
	B-B	147	144.602			
				2.842	1	.092
*MPI**						
	A-A	2	.762			
	A-B	20	22.476			
	B-B	167	165.762			
				2.294	1	.130
*PEPB**						
	A-A	10	5.845			
	A-B	47	55.310			
	B-B	135	130.845			
				4.334	1	.037
*PGM**						
	A-A	11	12.440			
	A-B	76	73.119			
	B-B	106	107.440			
				.300	1	.584

Appendix7.4: Tests for conformity to Hardy Weinberg equilibrium of pooled samples from spatial variation analyses.

Table 7.4.1: D231+F4 west coast spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	76	57.575			
	A-B	70	106.850			
	B-B	68	49.575			
				25.453	1	.000
*AH**						
	A-A	51	46.895			
	A-B	96	104.211			
	B-B	62	57.895			
				1.297	1	.255
*EST-4**						
	A-A	8	5.876			
	A-B	51	55.249			
	B-B	132	129.876			
				1.130	1	.288
*MPI**						
	A-A	0	.864			
	A-B	27	25.273			
	B-B	184	184.864			
				.986	1	.321
*PEPB**						
	A-A	8	5.510			
	A-B	53	57.979			
	B-B	155	152.510			
				1.593	1	.207
*PGM**						
	A-A	6	10.227			
	A-B	82	73.546			
	B-B	128	132.227			
				2.854	1	.091

Table 7.4.1: D4+F2+F3 West coast non-spawners

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	49	33.138			
	A-B	88	119.724			
	B-B	124	108.138			
				18.326	1	.000
*AH**						
	A-A	59	49.931			
	A-B	109	127.138			
	B-B	90	80.931			
				5.251	1	.022
*EST-4**						
	A-A	15	8.299			
	A-B	60	73.402			
	B-B	169	162.299			
				8.134	1	.004
*MPI**						
	A-A	5	1.321			
	A-B	27	34.357			
	B-B	227	223.321			
				11.876	1	.001
*PEPB**						
	A-A	6	4.990			
	A-B	61	63.021			
	B-B	200	198.990			
				.274	1	.600
*PGM**						
	A-A	12	10.560			
	A-B	81	83.879			
	B-B	168	166.560			
				.308	1	.579

Table 7.4.2: DUN231+F4+T143 western subpopulation

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	125	93.251			
	A-B	122	185.499			
	B-B	124	92.251			
				43.473	1	.000
*AH**						
	A-A	90	81.603			
	A-B	158	174.794			
	B-B	102	93.603			
				3.231	1	.072
*EST-4**						
	A-A	17	12.426			
	A-B	96	105.147			
	B-B	227	222.426			
				2.573	1	.109
*MPI**						
	A-A	1	1.145			
	A-B	39	38.710			
	B-B	327	327.145			
				.021	1	.886
*PEPB**						
	A-A	12	8.664			
	A-B	90	96.672			
	B-B	273	269.664			
				1.786	1	.181
*PGM**						
	A-A	15	19.267			
	A-B	140	131.467			
	B-B	220	224.267			
				1.580	1	.209

Table 7.4.3: CHB1+BB13 (CHBB)

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	65	40.852			
	A-B	57	105.297			
	B-B	92	67.852			
				45.021	1	.000
*AH**						
	A-A	53	41.153			
	A-B	83	106.694			
	B-B	81	69.153			
				10.701	1	.001
*EST-4**						
	A-A	23	16.653			
	A-B	70	82.693			
	B-B	109	102.653			
				4.759	1	.029
*MPI**						
	A-A	1	.978			
	A-B	27	27.044			
	B-B	187	186.978			
				.001	1	.981
*PEPB**						
	A-A	9	6.306			
	A-B	57	62.388			
	B-B	157	154.306			
				1.663	1	.197
*PGM**						
	A-A	25	20.617			
	A-B	85	93.767			
	B-B	111	106.617			
				1.932	1	.165

Levels of signficance from contingency chi-square tests between Cheynes Beach and the Esperance summer adults.

COMPARISON	*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
CHB1 v. ESP2+6	.818	.904	.007	.790	.989	.072

Table 7.4.4: BOSTON BAY, SA AND PPB VIC, (BSPP)

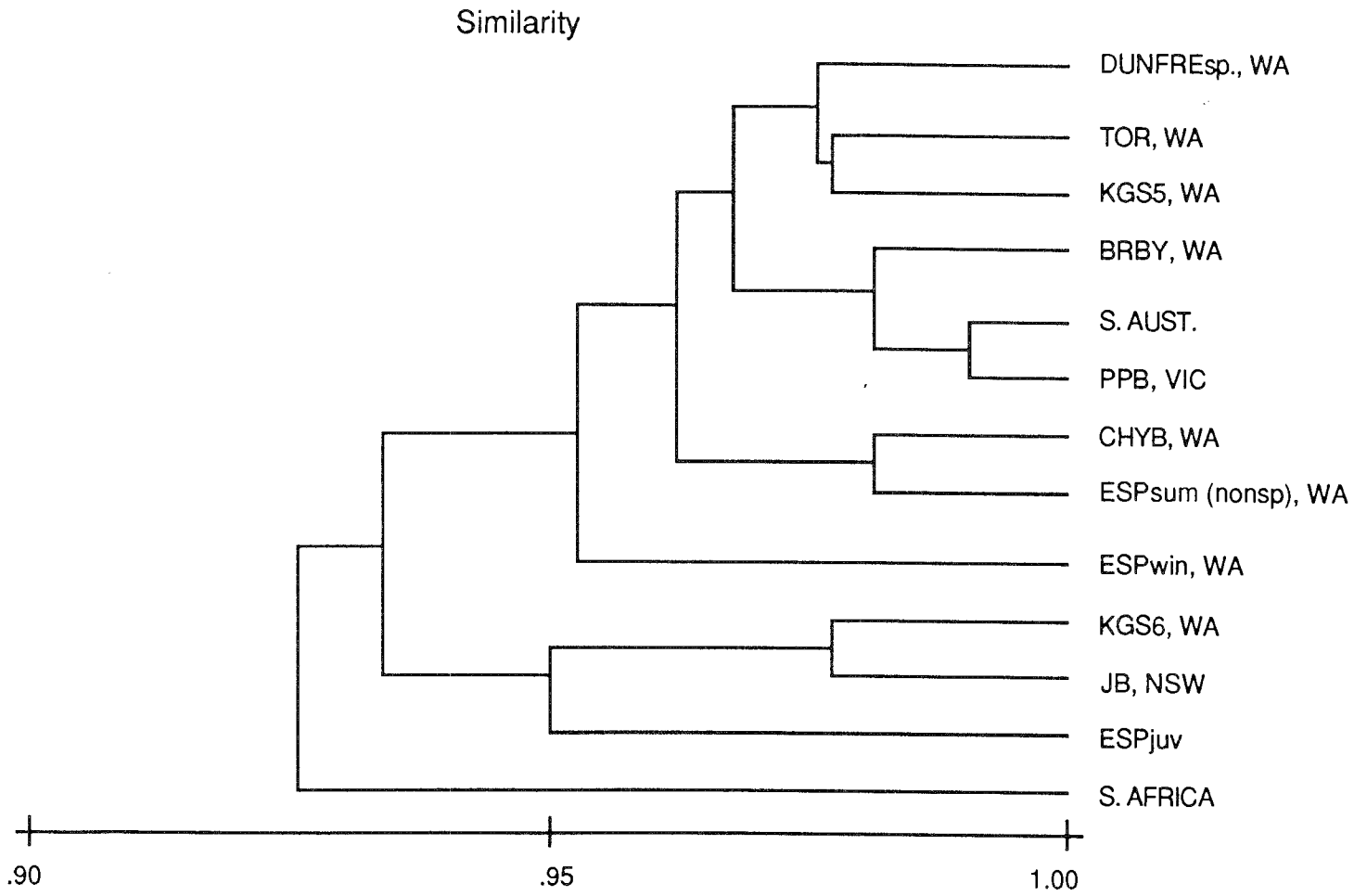
Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
*AAT**						
	A-A	69	49.271			
	A-B	99	138.458			
	B-B	117	97.271			
				23.146	1	.000
*AH**						
	A-A	74	65.607			
	A-B	125	141.787			
	B-B	85	76.607			
				3.981	1	.046
*EST-4**						
	A-A	6	2.880			
	A-B	45	51.239			
	B-B	231	227.880			
				4.181	1	.041
*MPI**						
	A-A	3	1.201			
	A-B	31	34.598			
	B-B	251	249.201			
				3.083	1	.079
*PEPB**						
	A-A	10	9.031			
	A-B	82	83.938			
	B-B	196	195.031			
				.153	1	.695
*PGM**						
	A-A	16	17.938			
	A-B	112	108.125			
	B-B	161	162.938			
				.371	1	.542

APPENDIX 8.0: *EST-4** Allele Frequency Distribution

*EST-4** allele frequencies categorised for each unpooled WA sample within their respective collection season.

	FREQUENCY CATEGORIES			
SEASON	< 0.7	>0.71-0.79	>0.8-0.9	>0.91
W '90	BB1, FM1	ESP1, DUN1	TOR1,KGS1 KGS2	
SP '90	KGS3		BB2, TOR2 FM2	
S '90	KGS4	BB3, DUN3 ESP2	TOR3, DUN2	
W'91	TOR4, FM3	BB4,KGS5	KGS6, ESP3 ESP4, ESP5	
S'91			DUN4, FM4 KGS7	ESP6

Appendix 9.0: Dendrogram of genetic inter-relationships of the Australian Pilchard.



Appendix 9: Dendrogram of genetic interrelationships of the Australian pilchard (*S. sagax neopilchardus*) and a sample of *S. ocellata* from South Africa, based on the analysis of 5 polymorphic loci (*Est-4** excluded) using Rogers' (1972) genetic similarity coefficient, as calculated using the UPGMA method, by BIOSYS-1. Site and sample codes are given in Table 10.
Goodness of fit statistics: Farris (1972) "f" = .957; Cophenetic correction = .717; Prager and Wilson (1976) "F" = 1.296;
Percent standard deviation (Fitch and Margoliash, 1967) = 1.948

APPENDIX 10.0: Comparisons between Esperance juveniles and other samples.

Appendix 10.1: Levels of significance of contingency-chi square comparisons between Esperance juveniles, other WA south coast groups and South Australia.

COMPARISON	*AAT**	*AH**	*EST-4**	*MPI**	*PEPB**	*PGM**
ESP3 v. DFT	.003	.124	.201	.345	.899	.022
ESP3 v. CHBB	.036	.359	.014	.246	.878	.313
ESP3 v. BSA	.116	.166	.300	.254	.717	.060