STOCK IDENTIFICATION AND DISCRIMINATION OF COMMERCIALLY IMPORTANT WHITINGS.

FINAL REPORT.

CENTRE FOR MARINE SCIENCE

UNIVERSITY OF
NEW SOUTH WALES

STOCK IDENTIFICATION AND DISCRIMINATION OF COMMERCIALLY IMPORTANT WHITINGS IN AUSTRALIAN WATERS USING GENETIC CRITERIA (FIRTA 83/16)

FINAL REPORT

By P.I. DIXON, R.H. CROZIER, M.BLACK and A.CHURCH
Centre for Marine Science and
School of Zoology,
The University of New South Wales

January 1987

TABLE OF CONTENTS

LIST OF FIGURES

PAGE

PAGE

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

SUMMARY OF MAIN FINDINGS

Sillago bassensis

The two sub-species of *S. bassensis* which were described by McKay (1985) are actually distinct species. Evidence in support of this conclusion is from three sources:

(a) Electrophoresis of liver and muscle enzymes. In these studies we found large numbers of fixed differences between the two subspecies; twelve out of the 43 loci were fixed for different alleles in the two forms. There was no evidence for introgression between the two forms at the only locality where they were sympatric (Anxious Bay, S.A.).

(b) Isoelectric focusing of soluble muscle proteins. Large differences were observed between the two sub-species when the patterns produced by their soluble muscle proteins were compared. These differences were also apparent in the specimens from Anxious Bay.

(c) Measurements of morphometric and meristic characters.
The differences that were observed in these characters were similar to those reported by McKay (1985). Discriminant function analysis was carried out on these data. There was no overlap of the confidence limits between the two sub-species; this separation supports the idea that the two groups have different phenotypes and is thus further evidence that these two groups of fish belong to two distinct species.

In this report we retain the terminology of McKay and refer to the two species as *S. bassensis bassensis* and *S.bassensis flindersi*.

Sillago bassensis flindersi.

We used seven polymorphic loci to investigate the population structure of *S. bassensis flindersi* throughout its known distribution. These studies revealed a large amount of population sub-structuring. However, the genetic relationships between the samples were not as expected on the basis of their geographic location: the samples were related in a haphazard way. This is thought to be due to patchy recruitment of larvae.

A discontinuity in the relatedness between samples was observed in the region between Forster and Coffs

Harbour. This discontinuity may indicate some degree of separation between the fish from northern and southern N.S.W. However, it is likely that a significant amount of gene flow occurs between them.

A high degree of genetic similarity was observed between the samples from southern N.S.W. and those from Victorian waters. Although it is possible that some degree of separation may occur between these samples, we have found no evidence to support this view. We believe that the fish from Victorian waters belong to the same sub-population as those from southern N.S.W.

Fish from Tasmanian waters are similar to those from the Lakes Entrance and San Remo areas. This may be the result of one-way flow of larvae across Bass Strait.

In South Australia, *S. bassensis flindersi* were obtained from Anxious Bay on the west coast. The allele frequencies in this sample were significantly different from those obtained in all of the other samples. These fish belong to a separate sub-population.

The distribution of *S. bassensis flindersi* extends from southern Queensland southwards to Tasmania and westwards to Anxious Bay in South Australia. Previously the recorded distribution of *S. bassensis flindersi* on the mainland was from southern Queensland to eastern Victoria (McKay, 1985).

Sillago bassensis bassensis.

The population structure of *S. bassensis bassensis* was investigated by means of electrophoresis of five polymorphic loci. The results of this very limited study suggested that each of the samples studies (one from W.A. and three from S.A.) may have been from separate sub-populations.

Discriminant function analyses of the morphometric and meristic characters of *S. bassensis bassensis* from Mandurah, W.A. and Spencer Gulf, S.A., support the idea that fish from these two areas belong to separate sub-populations.

We found the distribution of *S. bassensis bassensis* to extend from southern W.A. to St. Vincents Gulf and the western end of Kangaroo Island. It is possible, however, that it is distributed further eastwards. Further sampling is required to check this point. McKay (1985) believed that its range extends eastwards to San Remo.

Sillago robusta.

Liver and muscle enzymes of *Sillago robusta* from northern N.S.W., Rottnest Is. (W.A.) and Groote Eylandt in the Gulf of Carpentaria (N.T.) were compared using starch gel electrophoresis. Large differences were found between all samples from the three localities. In comparisons between fish from N.S.W. and W.A. fixed differences were observed at 13 of the 27 loci examined. There were 16/27 such differences when the fish from N.S.W. and N.T. were compared, and 7/27 in comparisons between the W.A. and N.T. fish.

Isoelectric focusing of the soluble muscle proteins of *S.robusta* revealed large differences between the fish from each of the three localities.

We believe that the N.S.W. sample of fish is a distinct species. It is also highly likely that the N.T. and W.A. fish belong to separate species. Further work should be done on fish from northwestern Australia to clarify this point.

Comparisons were made between samples of *S. robusta* from N.S.W. No major differences were found between the samples. We believe that these fish all belong to the same population and that the small differences that were observed are due to patchy recruitment.

Sillago maculata.

We compared samples of *S. maculata maculata* from N.S.W. and *S. maculata burrus* from Mandurah (W.A.) and Groote Eylandt (N.T.). The samples were compared at 23 enzyme loci but no fixed differences were observed.

Preliminary comparisons using isoelectric focusing did, however, reveal some small differences between fish from the three samples, but these have not been fully investigated.

It is unlikely that further work will reveal differences of the order of those found between samples of either *S.bassensis* or *S. robusta*. We believe McKay's sub-specific status for *S. maculata* is appropriate.

Sillaginodes punctata.

We compared samples of *S. punctata* from six localities in South Australia and Victoria. Evidence is presented that suggests a degree of population structuring in this species. However, the data set is small and

patchy; further work must be completed before conclusions can be reached.

Sillago vittata.

We used electrophoresis of liver and muscle enzymes and isoelectric focusing of soluble muscle proteins to compare *S. vittata* with *S. bassensis bassensis* and *S. bassensis flindersi.* The data obtained support the finding of McKay (1985) that *S. vittata* is a distinct species.

Sillago ciliata

We investigated 81 presumed gene loci in *S. ciliata.* Of the 23 loci which displayed polymorphism, 5 showed potential for future use in population comparisons in this species.

Sillago analis

The limited study on *S. analis* indicates that the level of polymorphism is relatively high with four out of the 12 loci studied showing polymorphism.

Our preliminary results suggest that, despite the morphological similarity between *S. analis* and *S. ciliata*, the genetic differences are considerable.

Sillago schomburgkii

In a small-scale pilot study we found that four out of the 15 loci we studied, in liver and muscle tissue, were polymorphic. Further work must be done to evaluate the potential for the use of these polymorphisms in studies on the population stucture of this species.

RECOMMENDATIONS

We make the following recommendations for the management of, and future research into, *Sillago bassensis flindersi*.

In New South Wales the main fishery is based at Yamba (Iluka) on the north coast, where the species is abundant. Smaller amounts are landed in other northern ports. The southern fishery is small and is based in Eden. The species has a patchy distribution between about Newcastle and Eden and few fish are landed in that area.

Lakes Entrance and San Remo are the main ports for landings in Victoria and in Tasmania there is a small fishery off the east coast.

We observed a discontinuity in genetic similarity between samples from the Forster to Coffs Harbour region of New South Wales. This suggests some degree of separation between fish from the northern and southern areas. There is, however, likely to be a significant amount of gene flow between them.

Recommendation 1

The fishery from about Newcastle north should be managed as a single unit.

In view of the likely gene flow between the northern and southern areas the fishery should be monitored carefully for signs of depletion in the southern areas.

We have no evidence for population subdivision in the region between Jervis Bay and Portland. There is some degree of separation between fish from the mainland and those from the Hobart area.

Recommendation 2

The fishery between Jervis Bay and Portland should be managed as a single unit. Thus consultation between managers from New South Wales and Victoria is essential.

The small fishery in Tasmania may be managed separately but should be monitored carefully as we know nothing of the extent of its possible reliance on a flow of larvae from mainland waters.

The sample from Anxious Bay in South Australia was significantly different from samples from all of the other areas that we examined. In South Australia,

school whiting are a trivial part of the by-catch of beach seiners. However, should a fishery develop two species are likely to be involved because we found that *S. bassensis bassensis* and *S. bassensis flindersi* are sympatric in South Australian waters. We have not been able to obtain samples of school whiting from the area between Kangaroo Island and Portland.

Recommendation 3

Sillago bassensis flindersi from west of Kangaroo island should be managed as a discrete unit.

However, preliminary indications are that *S. bassensis bassensis* from this area may not be a single unit.

Recommendations for Further Research

In view of the complex nature of the relationships between samples of this species, especially from the northern waters of New South Wales, further study is warranted. It is likely that the key to this complexity lies with larval ecology. The following matters deserve early attention.

1. Determination of the time of spawning. This should be done in at least ten localities on the east coast, e.g. San Remo, Lakes Entrance, Eden, Jervis Bay, Sydney, Forster, Camden Heads, Coffs Harbour, Yamba and Byron Bay. This study should include studies on the Gonadosomatic Index (by month) and histological examination of gonad development. We understand that such a study is in progress.

2. Determination of the location of spawning on the continental shelf and the length of larval life.

3. Investigations into the hypothesised patchiness of larval distribution and the genetic relatedness of larvae from different patches of water.

Such a study would be a major undertaking but is likely to yield fundamental information about recruitment in this and other species. Sampling could be carried out along a transect and be followed by identification of the water mass from which the individual samples were obtained. However, it would be preferable to carry out such a study from a ship with real time access to NOAA satellite images. Under these conditions individual water pockets could be identified accurately and sampled. Genetic relatedness of larvae from the different water pockets could be identified by gel electrophoresis techniques.

Because of the extent to which the fishery for *Sillago robusta* impinges on *S. bassensis flindersi* in northern N.S.W. we recommend that the same data be obtained for that species.

With regard to *S. bassensis bassensis* in South Australia, further investigations into the population structure of this species should be made. This matter is discussed in the body of the report.

ACKNOWLEDGMENTS

During the course of this large programme there have been many people who have helped us in many different ways. We are most grateful to them all.

Special thanks are due to those who have made collections of samples for us, especially David Smith (NSW Fisheries Research Institute), Jacek Wankowski (Marine Science Laboratories, Queenscliff), Keith Jones (S.A. Department of Fisheries), and Rod Lenanton and Mark Cliff (W.A. Fisheries Laboratories) who have provided us with numerous samples. Others who have provided us with samples include: Kim Evans (Tasmanian Fisheries Development Authority), Neville Gill (N.T. Department of Ports and Fisheries), Rob Howard and Jock Young (C.S.I.R.O. Division of Fisheries), Peter Hall and Gary Henry (N.S.W. Fisheries Research Institute) and Clive Keenan (University of Queensland).

Our thanks also go to Richard Holliday, computer programmer and odd-job man, and to those Marine Ecology students from the classes of 1984-1986, and others who helped with sample collection and tissue preparation, and to all those who unsuccessfully tried to obtain samples on our behalf.

INTRODUCTION

Whiting are small to medium sized fish which inhabit the shallow coastal waters of the Indian and western Pacific Oceans. They belong to the Family *Sillaginidae*. This Family has recently been reviewed by McKay (1985). It consists of three genera, *Sillago*, *Sillaginopsis* and *Sillaginodes*. McKay considers that there are three sub-genera of *Sillago*, viz. *Sillaginopodys, Parasillago and Sillago*. Ten of the 25 species which make up this family are found in Australian waters. These are *Sillaginodes punctata, Sillago analis, Sillago bassensis, Sillago ciliata, Sillago lutea, Sillago maculata, Sillago robusta, Sillago schomburgkii, Sillago sihama* and *Sillago vittata* (see Appendix 1). Many of these species are morphologically very similar.

The flesh of all species has a fine texture and a delicate flavour which is retained after freezing. Many of these species are sought after by recreational fisherman and, over their whole distributional range, whiting form the basis of small fisheries of commercial importance.

The following brief description of the species with which we have worked during this study are based on McKay's (1985) review, where further details may be found.

Sillago ciliata (sand whiting) is a very good eating fish which grows to about 50cm in length. It moves in large schools across sand banks, and in the surf zone. It is a common angling fish and is of commercial importance in New South Wales. It is a silvery white fish with unblotched sides but with a distinctive dark blotch at the base of its pectoral fin. The fins are yellow except for the dorsal which is pale green. McKay says it is distributed throughout Eastern Australia (Figure 1a).

Sillago analis (golden-lined or rough scale whiting) is very similar to *S. ciliata* and the two species are found together in sandy estuaries in Queensland. It grows to about 30cm in length and has a silvery coloured body which is slightly darker dorsally, with a yellow band just below the lateral line. The fins are yellow but there is no black spot at the base of the pectorals which have a fine dusting of brownish spots. McKay considers that *S. ciliata* and *S. analis* are sibling species: there are suggestions that these two species sometimes hybridise in nature. *Sillago analis* is found in Northern Australia from Moreton Bay (Qld) to Shark Bay (W.A.), see Figure 1b.

Sillago bassenis (school or red-spot whiting) is regarded by McKay as a single species, but he found differences between the eastern and western forms, namely in second dorsal and anal fin ray counts, numbers of lateral line scales and

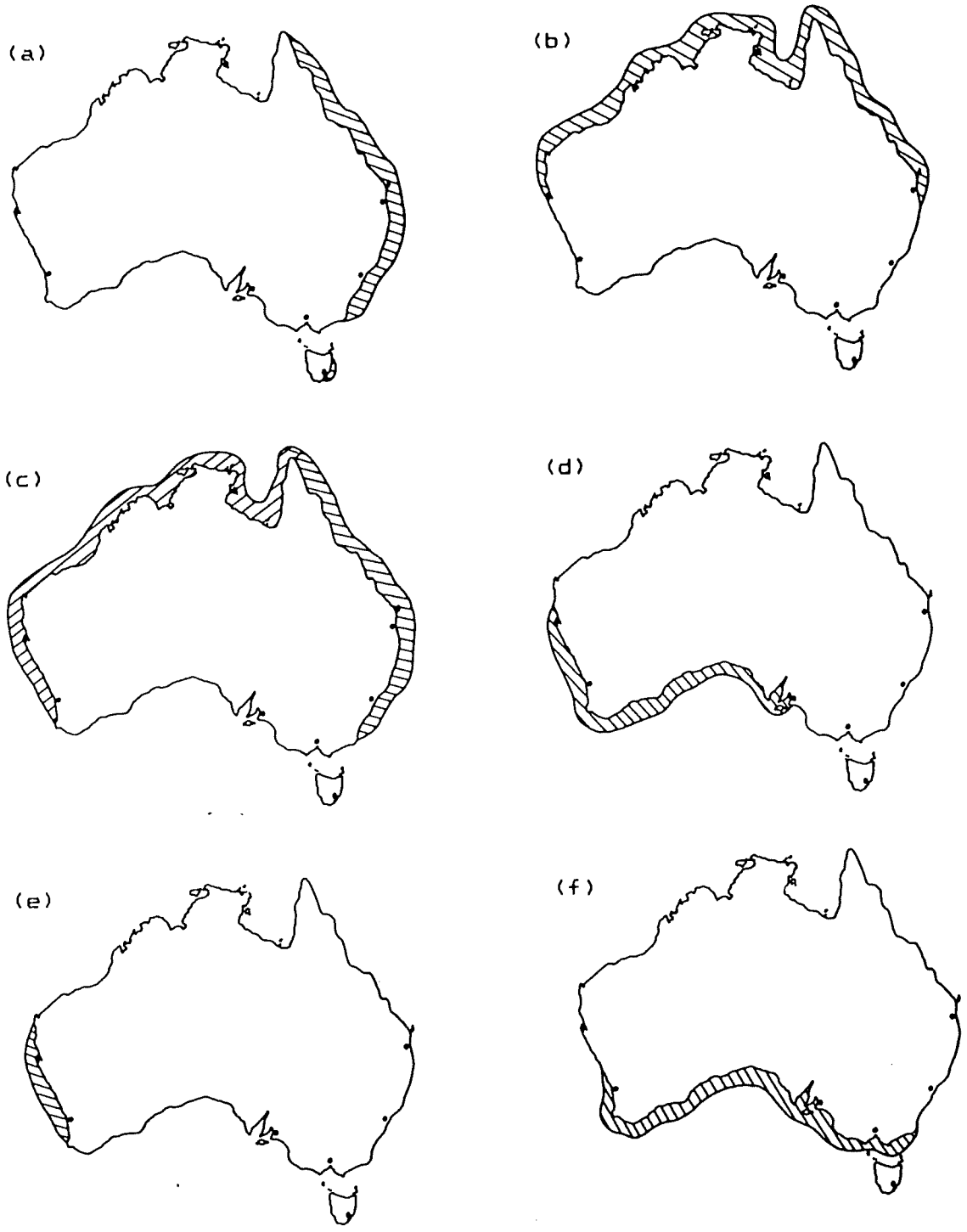


Figure 1: Maps to show the distribution of (a) *S. ciliata*, (b) *S. analis*, (c) *S. maculata*, (d) *S. schomburgkii*, (e) *S. vittata* and (f) *S. punctata* after McKay (1985).

numbers of vertebrae. McKay found, however, overlap between the two forms in fin ray counts and numbers of lateral line scales. The total number of vertebrae in each form is distinct, but, when the different kinds of vertebrae (e.g. abdominal, caudal etc.) are considered there is a great amount of overlap between the two forms in the numbers of each type of vertebra. Both forms grow to about 32cm in length.

There are also distinguishing colour and pattern differences between the two forms. These differences are apparent in fresh and frozen specimens that have not suffered scale loss. These differences are:

(i) The western form has oblique rusty brown bars on its upper body. These bars are often broken into oblique rows of dots or blotches. This fish has a distinct silvery mid-lateral line, but there are no rusty brown blotches mid-laterally. The belly is pink or white, and the pectoral fin is pale cream, without a dark blotch near its base.

(ii) The eastern form has oblique rusty red to bright orange broken and unbroken bands above the lateral line, and a series of about 12 similarly coloured blotches just above an obvious silvery lateral band. The oblique bands are more regular, and broader, than those in the western form. The belly is pale silvery white, the pectoral fin a dull yellow, without a dark blotch at its base.

McKay places much importance on swim bladder morphology as a means of distinguishing between the species of whiting.

He found no differences in swim bladder morphology between the eastern and western forms of *S. bassensis*. He regards the two forms as sub-species which he found to have the following distributions:

(i) *S. bassensis bassensis* is the western form, and is found from western Victoria across the southern coast of Australia and northwards to Geraldton in Western Australia.

(ii) *S. bassensis flindersi* is the form that occurs in Queensland, New South Wales, eastern Victoria and eastern Tasmania (see Inset, Figure 8).

Sillago robusta (stout whiting) is a smallish creamy yellow whiting which grows to about 28cm in length. It has a silvery band along its side and a yellow blotch on its cheek. McKay describes two forms of this species, an eastern form which extends along the each coast of Queensland to southern New South Wales and a western form which is distributed from Fremantle northwards to the Gulf of Carpentaria (see Inset, Figure 21).

Sillago maculata (trumpeter whiting) has a pattern of dark blotches on a silvery body. There is also a dark blotch at

the base of the pectoral fin. McKay describes three sub-species which are all of similar size (they all grow to about 30cm), colouring and morphology. Two of the sub-species occur in Australian waters; *S. maculata maculata* extends along the east coast of Australia and *S. maculata burrus* occurs on the northern and western coasts of the continent (Figure 1c). The main diagnostic features are differences in swim bladder morphology.

Sillago schomburgkii (yellow-fin whiting) is a very important recreational fish in South Australia and Western Australia. This fish has a silvery appearance and yellow fins. It is very similar to *S. ciliata* in appearance and habitat requirements but has no dark blotch at the base of the pectoral fin and grows to a smaller size (about 40cm in length). Its distribution is shown in Figure 1d.

Sillago vittata (western school whiting or banded whiting) is a newly described species which has only been recorded from Western Australia. Its known distribution is from Maud Landing to Mandurah (Figure 1e). Its maximum recorded length is 30cm. No geographic variation has been observed in this species. It is often found in asssociation with *S. robusta, S. bassensis bassensis* and *S. maculata burrus*. In the north it is found in shallow waters but in southern Western Australia it is usually trawled in deeper waters (17-20 fathoms).

Sillaginodes punctata (King George whiting) is the largest of the whitings; it grows to about 70cm in length. It is easily distinguished from all other species by means of the rows of small dark brown to rusty brown spots which occur on its back and upper sides. It is distributed from Jurien Bay (W.A.) across the south of the continent to southern New South Wales (Figure 1f). It inhabits sheltered coastal bays and rocky reefs. It spawns outside these bays but the larvae are carried back into them and the juveniles grow in mangrove and seagrass nursery areas (Jones, 1981). This species is the basis of important commercial fisheries in Victoria, Western Australia and especially South Australia.

We originally proposed to investigate the population structure of three species of whiting from eastern Australia. These were: *S. ciliata, S. bassensis* and *S. punctata*. These are the most important species commercially. The King George whiting fishery is worth $A2 million annually in South Australia alone (Jones, 1980). In New South Wales the sand whiting fishery returns about $A500,000 (Table 1). Although the sales of red spot whiting through the New South Wales fish marketing authority are relatively low (Table 1) an important export market has developed for this species (Table 2) which last year returned over $A2 million.

The King George and sand whitings spend a significant part

Year	Species	Quantity kg	Average Price $A per kg
1978-9	Red-spot	164,707	0.8
	Sand	94,551	3.38
	Trumpeter	26,333	1.56
1979-80	Red-spot	109,810	1.08
	Sand	108,857	3.74
	Trumpeter	29,294	1.73
1980-81	Red-spot	110,503	1.09
	Sand	151,907	3.09
	Trumpeter	42,274	1.44
1981-82	Red-spot	148,911	1.10
	Sand	138,255	4.20
	Trumpeter	37,601	1.51
1982-83	Red-spot	242,798	0.95
	Sand	159,814	4.45
	Trumpeter	44,587	1.57
1983-84	Red-spot	318,077	0.77
	Sand	154,190	4.58
	Trumpeter	41,107	1.56

TABLE 1: Whiting sold via NSW Fish Marketing Authority for years 1978-1984. Data from N.S.W. Fish Marketing Authority, Annual Reports 1979-1984.

Year	Tonnes	$A
1980-81	777	878,000
1981-82	1,499	1,885,000
1983-84	1,091	1,173,000
1984-85	1,042	1,396,000
1985-86	1,347	2,577,000

Table 2: Australian whiting exports for years 1980-1986. Data from "Australian Fisheries".

of their lives in estuaries or sheltered embayments and it was during that stage of their life histories that we intended to obtain samples of these two species. The red-spot whiting is not usually found in large numbers in estuaries; it was not therefore our intention to investigate this species fully as we expected difficulties in obtaining samples.

Other species were also to be investigated but to a lesser extent. This was again mainly because of the difficulties, to us, in obtaining samples. These other species included *S. robusta, S.maculata* and *S. analis*.

The major objective of our programme was to investigate the population structure of commercially important whitings using allozymes as genetic markers. Electrophoretic methods were to be used to determine whether each species is characterised by one large interbreeding population throughout its range, or whether it is made up of two or more sub-populations with some degree of isolation. Where evidence of sub-populations is found the geographic limits of 'stocks' were to be determined. Such information is important in considerations on the rational management of the stocks.

With this in mind, during the first few months of the programme, we carried out pilot studies on each of the species mentioned. However in May 1984, at the request of the South Eastern Fisheries Committee (SEFC), Demersal and Pelagic Fish Research Group, the project changed in emphasis. This group expressed concern at the lack of knowledge of the biology and population structure of *S.bassensis*. Such information was urgently needed because of the developing fishery for this species. In New South Wales *S.bassensis* and *S. robusta*, which make up about 10% of the red-spot catch, were trash fish. They were part of the by-catch of prawn trawlers (Bowerman, 1984) until the late 1970's when an export market to Japan was developed, and a Ministerial concession allowed prawn trawlers, working north of Smoky Cape, to land whiting. This northern part of the range of the species accounts for about half of the current landings (Hobday and Wankowski, 1986). In the south there are fisheries for red-spot whiting centred on Eden, Lakes Entrance and San Remo. Table 2 gives the details of exports from 1980-1986. The development of the fishery for school whiting in Victorian waters was reviewed by Winstanley (1983).

Prior to the development of the export market little interest was shown in this species, so virtually no background information was available. Fisheries researchers from each of the states represented in the group (N.S.W., Vic., S.A. and Tas.) agreed to obtain samples for us so that we could direct our major effort towards gaining an understanding of the population structure of *S. bassensis*. At the same time they would collect information on catch and

effort, age and growth, population movements, natural and fishing mortality and reproduction. The implications of these data for management are considerable. Unless there is sub-structuring of the red-spot whiting population, management will have to be on a regional rather than on a State or local basis. Such management will have to consider the impact of each fishery on the other.

The major part of this report presents our findings on the population structure of *S. bassensis*. Other subsiduary findings on *S. robusta, S.vittata, S.maculata, S.ciliata, S.schomburgkii* and *S.punctata* are also presented.

8

METHODS

SPECIMEN COLLECTION

Specimens were collected with the cooperation of the Fisheries Division (Dept. of Agriculture) and various other institutions in each state. We obtained samples of *S. punctata, S. analis, S. bassensis, S. ciliata, S. maculata, S. robusta, S. schomburgkii* and *S. vittata*. Refer to Tables 2.1 to 2.9, in Appendix 2 for details of these collections.

Fish caught by us were entrapped using beach seine nets in various lakes and bays. For *S. ciliata* and *S. maculata*, a net of 100m in length, with a mesh size of approximately 30mm at the cod end, was used. For *S. punctata*, a net of 25m in length, with a mesh size of approximately 12mm, was used. Some of the fish sent to us were caught using hand lines, but the bulk of the fish were caught by prawn trawlers.

Specimens of *S. bassensis flindersi* and *S. robusta* in NSW were caught on cruises conducted by the NSW Fisheries Research Institute (refer to Kapala Cruise Report Nos: 94, 97), or obtained from Fishermen's Co-operatives. They were frozen as soon as possible after collection.

Fish caught by beach seine were transported back to the laboratory on ice. Upon arrival, these fish were stored frozen at -20°C. Those fish caught by prawn trawlers were frozen on board.

TISSUE PREPARATION

Fish were partially thawed and measured for standard length (S.L.) and length to caudal fork (L.C.F.), sexed (with note of gonad condition), and samples of particular tissues taken for electrophoresis. For the pilot study, samples of liver, "white" skeletal muscle, heart and eye lens were screened for tissue specificity of enzyme loci. However, for the bulk of the study, liver and muscle proved to be the most useful tissues. All tissue samples were stored in 1.8ml Nunc cryotubes in liquid nitrogen (-180°C to -196°C) until required for electrophoresis.

Tissue samples were partially thawed and homogenized with an equal volume of cold deionized water or homogenizing buffer (see Table 4.2 in Appendix 4) using a perspex rod. Tough tissues (muscle, heart, eye lens) were finely minced with scissors prior to

homogenization. For the enzymes used in stock discrimination of *S. bassensis* and *S. punctata*, we found that homogenizing buffer gave better resolution on the gels. For *S. robusta*, water gave better resolution. For isoelectric focusing, "white" skeletal muscle was homogenized in cold deionized water, to minimize the salt load of the samples.

Homogenates were centrifuged in an MSE Mistral 6L refrigerated centrifuge for 20 minutes, 2000 r.p.m. (1000 x g), at 4°C. Samples were then stored frozen at -20°C and electrophoresed during the same week of preparation.

Due to the amount of free oil separated from the liver samples of *S. bassensis*, it was necessary to carefully draw off the supernatant (from under the plug of fat and oil) with a pasteur pipette and place into 1.5ml Eppendorf tubes. Free oil was not a problem with any of the other species studied, and the frozen fat plug (when present) was removed with a spatula to expose the supernatant. Several unsuccessful attempts were made to extract oil from the school whiting liver samples (Carbon tetrachloride, Butylacetate, Toluene). It was found that this extra step in sample preparation either destroyed enzyme activity on the gels, or did not improve the resolution. For *S. bassensis flindersi*, extraction buffer (see Table 4.2, Appendix 4) was also tried, to release membrane-bound proteins into the supernatant. However, due to the excessive amounts of fat in the liver of this species, the supernatant was of high lipid content, resulting in streaking of bands on the gels.

ELECTROPHORESIS

Various support media were investigated for their usefulness, and are detailed below. For the bulk of the study, it was found that starch was the most suitable system.

Cellulose acetate separates proteins by net charge alone, whilst starch also has a "molecular sieving" effect, thus separates proteins by size as well as net charge. This "molecular sieving" may be beneficial, or may (at times) mask variation due to net charge. We have also found starch to be more sensitive than cellulose acetate with lower concentrations of protein staining on starch whereas there was no activity on cellulose acetate.

Prior to the commencement of population comparisons, pilot studies were carried out on all of the species collected. The strategy used was essentially the same as that described by Richardson *et al.* (1986). One or,

if possible, two populations of each species was used to determine which enzymes displayed polymorphism. The enzymes studied in each species are listed in Tables 3.2 to 3.11, Appendix 3. Those loci which were polymorphic formed the basis of population comparisons in species where these were made.

Those loci that are monomorphic with different alleles in different species allow definitive tests for the occurence of hybridisation. The proportion of loci differing between species also contributes most of the information needed for species identification and for inferring relationships between species.

Starch Gel Electrophoresis

Horizontal starch gel electrophoresis was carried out at 5°C in a 12% (w/v) Electrostarch gel. The various buffer systems used are listed in Table 4.1 of Appendix 4. Samples were located onto the gel using sample strips (cut from Whatman #3 filter paper) wetted with supernatant prepared as described previously. A total of 25 samples, and 2 standards, could be run on each gel. After electrophoresis, the gel was sliced (into 3 or 5 pieces) with each slice being treated with an enzyme-specific histochemical stain. Table 3.1, Appendix 3 lists the enzymes used in this study. See Table 4.4, in Appendix 4, for details of the staining recipes followed. Staining reactions were incubated at 37°C in the dark for 3 minutes to 1 hour, depending upon the enzyme being investigated. Staining reactions were stopped with fixative (Table 4.6, Appendix 4) and scored. A photographic record has been kept for all stock discrimination work.

Cellulose Acetate Gel Electrophoresis

Commercial preparations of Cellogel and Titan III cellulose acetate plates were investigated for the separation of some enzyme loci in some fish species, as indicated in Appendix 3. The cellulose acetate was prepared for electrophoresis by soaking in the appropriate buffer to equilibrate. The various buffers used are listed in Table 4.1, Appendix 4. Up to 4ul of sample was applied to the surface of the gel; 10 samples and 1 standard could be run on each gel. After electrophoresis, the gel was treated with an enzyme-specific histochemical stain. See Table 4.5, in Appendix 4, for details of the staining recipes followed. Staining reactions were incubated at room temperature in the dark for 3 minutes to 30 minutes, depending upon the enzyme being investigated. Staining reactions were stopped with fixative. The gels were scored by marking the position of the bands on the

plastic backing of the gels. Cellogel may be stored wrapped in plastic in the freezer. Titan III plates may be stored dried.

Isoelectric focusing

LKB Ampholine polyacrylamide gels (pH range 3.5 to 9.5) were used to separate soluble muscle proteins for a comparison of each species (and sub-species) studied. Gels were prefocused to 500 Volthours, prior to loading of the samples, to set the isolines. This step proved necessary to minimize the waving of bands due to the salt load of the samples. After electrophoresis, the gels were fixed, according to LKB instructions, and stained for general protein, with Page Blue 83, for 2 hours. The gels were destained overnight and covered with plastic film for storage.

Agarose I.E.F. (pH range 3 to 10 Pharmalyte) was attempted for two polymorphic loci of King George whiting (as indicated in Table 3.1, Appendix 3). However, the results were unsatisfactory, and this system was not investigated further.

ELECTROPHORETIC DATA ANALYSIS

Patterns of enzyme variation that were consistent with the known subunit structure of the enzyme (Shaklee and Keenan, 1986) were used for discrimination of stocks. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Commission on Biochemical Nomenclature (Anon, 1984). For multilocus enzyme systems, the form with the least anodal migration was designated "1", the next "2", and so on (in accordance with the recommendations of Allendorf and Utter, 1979). For each locus, alleles were indicated alphabetically, with the most anodally migrating allele designated "a", the next "b", and so on. For loci with cathodal migration, the most cathodally migrating allele was designated "a". The putative genotype data were tabulated as genotype and allele frequency distributions, for each species, in a form suitable for input into the statistical programs described below.

We used Felsenstein's (1981, 1982) continuous character, maximum likelihood method for constructing phylogenetic trees from these data. The program CONTML (Version 2.7) is part of Felsenstein's PHYLIP package. See Appendix 5 for further explanation of this program and its assumptions. The program, CONPLOT, written by us, uses the output from CONTML to plot a dendrogram.

Dendrogram construction provides valuable information on the inter-relationships of populations, but does not provide a test of whether pairs of populations are

genetically distinct. The G-test (Sokal and Rohlf, 1981, pp 745-746) provides a simple, yet powerful test for distinguishing populations, and uses all the gene frequency data available. The program POPSEP, written by us, performs G-tests on all possible pairs of populations.

The genetic distance between pairs of populations was also used to construct phylogenetic trees. This was done, not because it is the most appropriate method, but because of the widespread application of these measures in electrophoretic studies of systematics (Hillis, 1984). The program NEISTAT, written by us, computes Nei's genetic distance, D* (as modified by Hillis, 1984), from the allele frequency distributions. The program NEISTT1, computes D* and tabulates in a form suitable for input into the following statistical program.

We used Felsenstein's (1981-1982) Fitch-Margoliash least-squares distance method for constructing phylogenetic trees from these data. The program FITCH (Version 2.8), is part of Felsenstein's PHYLIP package (see Appendix 5 for details of this program). The program FITPLOT, written by us, uses the output from FITCH to plot a dendrogram.

The genotype distributions of various loci in each species were examined for internal consistency with the Hardy-Weinburg distribution. The program G-FIT, written by Dr. D. Croft (School of Zoology, UNSW), uses G-tests to check the goodness-of-fit of observed genotype ratios with those expected for a single, randomly mating population (in the absence of differential selection among alleles). These selected genotype data were then analysed using POPSEP to test whether pairs of populations were significantly different. This method has the advantage over other tests (e.g. F statistics) which are commonly used in that it allows all the available data to be used.

We used Felsenstein's (1981,1982) mixed method parsimony to construct dendrograms from the isoelectric focusing data. The program MIX (Version 2.8) is part of Felsenstein's PHYLIP 5 package. The gels were scored for each species as a series of two state characters ("1" and "0") to indicate presence or absence of a band, respectively. For more details on this program see Appendix 5.

MORPHOMETRIC AND MERISTIC MEASUREMENTS

Nine morphometric measurements and eight meristic counts were made on 496 school whiting (approximately 100 individuals from each of five geographical regions of Australia). These regions represent relatively

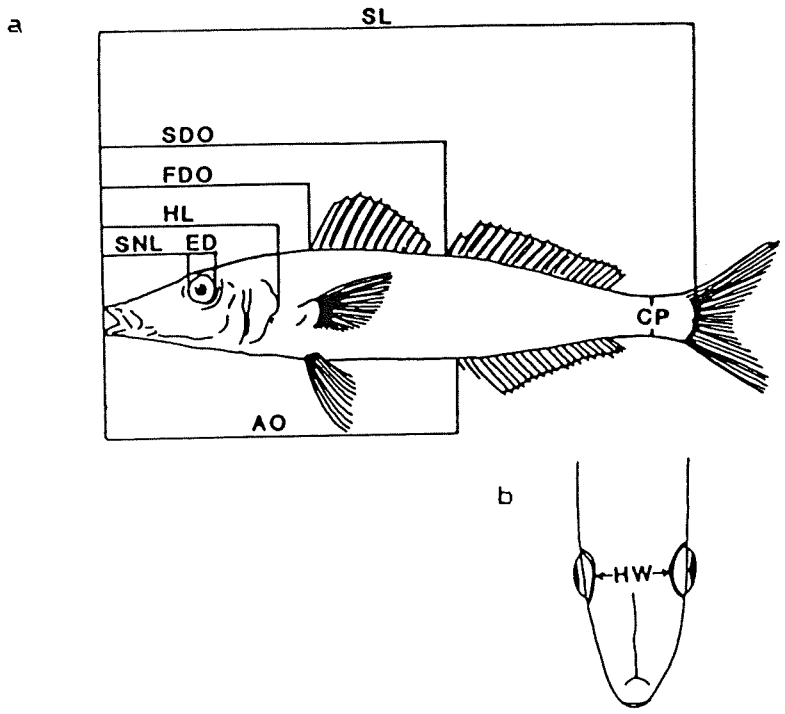


Figure 2: Morphometric measurements from *S. bassensis*. (a) lateral view, (b) dorsal view of head. The variables measured are defined in the text.

discrete and homogeneous geographical areas that are delimited by major hydrological features. These areas were as follows: Yamba, NSW; Mandurah, WA; Hobart, Tas; Eden, NSW and Spencer Gulf, SA.

Meristics

The dorsal and anal fin spines and rays were counted. The last dorsal and anal fin pterygiophore normally supports two rays which were counted as a single element in accordance with McKay (1985).

Lateral line scales bearing pores were counted from the upper margin of the operculum to the caudal flexure at the posterior margin of the hypural. Transverse scale rows were counted from the origin of the dorsal fin in a posterior oblique row to, but not including, the lateral line scales, and from the origin of the anal fin obliquely forwards and upwards to the lateral line scales.

Morphometrics

The nine morphometric measurements (Figure 2) were made along the longitudinal axis of the body using a fish measuring board for standard length (SL) measurements. Other measurements were made using a pair of digital calipers connected to a personal computer via an interface (Griffiths *et al*, 1986). Standard length was measured to the nearest millimetre, all other measurements were made to the nearest 0.01mm. Details of measurements were as follows:

Standard Length (SL): from the tip of the snout from the upper lip to the caudal flexure at the hypural margin.

Snout to first dorsal fin (FDO): from the tip of the snout to a line perpendicular to the origin of the spinous dorsal fin.

Snout to second dorsal fin (SDO): from the tip of the snout to a line perpendicular to the origin of the spine preceding the rayed second dorsal fin.

Snout to anal fin (AO): from the tip of the snout to a line perpendicular to the origin of the first anal spine.

Caudal peduncle (CP): least depth of the caudal peduncle.

Head length (HL): from the tip of the snout to the posterior margin of the fleshy operculum but anterior to the operculum spine.

Head width (HW): the least width of the bony interorbital space.

Eye diameter (ED): the horizontal diameter between the fleshy margins of the orbit.

Snout length (SNL): from the tip of the snout to the anterior fleshy margin of the eye.

Morphometric and Meristic Data Analysis

Various univariate transformations have been advanced for altering or removing size information from data (Reist, 1985). Three transformations were applied here for comparative purposes using multivariate discriminant analyses.

Proportional measures: A standard technique in systematic studies is the creation of a ratio (or proportion) between each of the variables (Y) and some standard size measure (X). The shape estimate (R) for an individual is then

$$R = Y/X \qquad (1)$$

For each region ratios were created between the variables HL, FDO, SDO, AO, CP and SL, yielding the shape variates HDSL, FDSL, SDSL, ANSL and CASL; the remaining three variables HW, ED and SNL were taken as a proportion of HL, yielding the variates HEHL, EYHL and SNHL. These ratios are consistent with those produced by McKay (1985).

It has been suggested that ratios do not completely remove the influence of size variation from the data (Dodson, 1978; Albrecht, 1978; Atchley et al., 1976). Hills (1978) argued that many of the problems with ratios result from nonlinear relationships between the ratio and the original variables and suggested that such problems may be alleviated by taking the logarithm (log) of the ratio. That is,

$$R = \mathrm{Log}\,[(Y)/(X)] \qquad (2)$$

Size-related measures: Thorpe (1975) developed an allometric formula for adjusting variables to those expected for a mean body size:

$$R = \mathrm{Log}Y - B(\mathrm{Log}X - \mathrm{Log}M) \qquad (3)$$

Here, Y is the original unadjusted measurement, B is the allometric coefficient (the slope of the relationship between log Y and log X), X is the standard length (SL) or head length (HL) of the individual, M is the grand mean SL or HL across all individuals from all regions, and log is the base-10 logarithm. Thus, these shape variates are predictions of what an individual's size for a particular variable would be if that individual was the overall mean standard length or the overall mean head length.

Thus, through the use of the appropriate transformations, three data sets descriptive of shape were created: the raw measurements divided by SL and HL (RATIO); the base-10 logarithms of these ratios

(LGRATIO); and the allometrically adjusted measurements (ALLOM). The efficiency of these transformations in removing the influence of size variation was examined by simple least squares linear regression of the shape variate on the appropriate size variable (SL or HL) and by testing the null hypothesis that the slope equalled zero. Another indication of the degree of the relationship and thus the ability of the shape variate to be free from the influence of size variation is provided by the squared correlation coefficient (R^2). The effects of transformations on normality was also investigated using the techniques of Sokal and Rohlf (1981,p139).

Differences in biological interpretations of the covariance (dispersion) structure of the various transformations were evaluated by direct discriminant analysis (Nie et al., 1975). The five group centroids on the four possible discriminant functions were given an isodensity circle containing 90% of all cases for each group centroid using the technique of Dillon and Goldstein (1984). The group centroids were clustered to determine the similarity of the discriminant analyses solutions to each other using the unweighted pair group method centroid (UPGMC). Discriminant analyses were performed using SPSS version 8.3 and cluster analyses using SPSS-X release 2.1.

Plate 1. *Sillago bassensis bassensis*

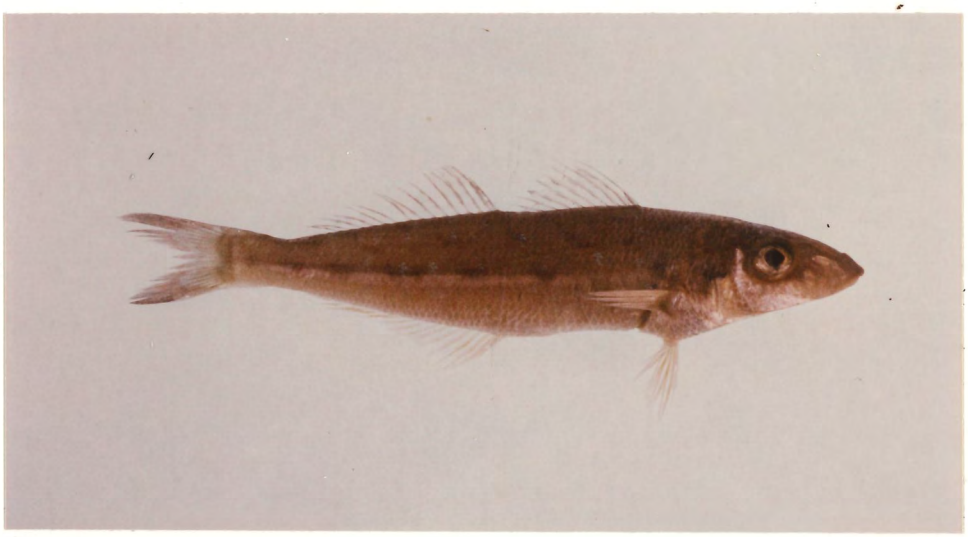


Plate 2. *Sillago bassensis flindersi*

Plate 3. *Sillago vittata*

RESULTS AND DISCUSSION

Sillago bassensis.

When our study began, school whiting, *Sillago bassensis*, were thought to be widely distributed around the continent, extending from southern Queensland southwards along the coast to eastern Tasmania from western Victoria westwards to Western Australia and up the west coast to about Geraldton.

While McKay was reaching the conclusion that *S. bassensis* was made up of two sub-species (*S. bassensis bassensis*, the western form and *S. bassensis flindersi*, the eastern form) we obtained specimens of *S. bassensis* throughout its range. Our prime concern was to investigate the population structure of *S. bassensis* in the area under the control of the South Eastern Fisheries Committee (SEFC).

Electrophoretic Studies

We began with a pilot study which included a sample from each end of the area under the control of SEFC, namely Yamba, N.S.W. and St. Vincents Gulf, S.A. We investigated 44 enzymes which encode for 75 presumed genetic loci. There were 19 suspected polymorphic loci. These were: Aat-2, Ada, Adh-1, Ald-1, Cat, Damox, Est, Gpi-1, Gpi-2, Gpi-3, Idh-1, Idh-2, Me-2, Mpi, Pep-C, Pep-D, Pgd, Pgm-1, Pgm-2 (see Tables 3.3 and 3.4, Appendix 3).

We noticed differences in the appearance of the fish from the two localities, but more importantly there appeared to be major genetic differences. We therefore carried out a detailed comparison of these two forms, for which we use McKay's terminology of *S. bassensis bassensis* and *S. bassensis flindersi*. We used starch gel electrophoresis of liver and muscle enzymes, and isoelectric focusing of soluble muscle proteins to compare the two forms of *S. bassensis*. We also included *Sillago vittata*, a newly described western species which has a superficial resemblance to *S. bassensis* (see Plates 1-3).

We examined liver and muscle enzymes (27 different enzymes) in these fish by starch gel electrophoresis. Because some of these enzymes occur in more than one form, some of which are products of different genetic loci, the 27 enzymes represent 43 presumed loci.

We found large differences in comparisons between the different whiting. Table 3 gives the details of the differences that were found to be fixed in species pair

Enzyme	Tissue	Number of Loci	SPECIES PAIR DIFFERENCES		
			BASS/VITT	BASS/FLIN	VITT/FLIN
AAT	L	2	Aat-2	Aat-2	Aat-2
ADA	L	1	Ada	Ada	Ada
ADH	L	2	Adh-1	Adh-1	
ADK	L&M	2			
ALD	M	1			
CAT	L	1			
CDA	M	3			
DIA	L	2	Dia-2	Dia-2	Dia-2
EST	L	2			
FUM	M	1			
GDA	M	1			
GOX	L	1			
GPI	L&M	4	Gpi-1(M)	Gpi(-L);Gpi-1(M)	Gpi-1(M)
GPT	L	1	Gpt		Gpt
IDH	L&M	2	Idh(-L)	Idh(-L)	Idh(-L)
LDH	M	1			
MDH	L&M	2		Mdh-1;Mdh-2	Mdh-1,Mdh-2
ME	L&M	2	Me(-L)		Me(-L)
MPI	M	1			
PEPB	L	2	PepB-1	PepB-1;Pep B-2	PepB-1;PepB-2
PEPC	L	2	PepC-1		PepC-1
PEPD	L	1	PepD	PepD	
PGD	L	1			
PGM	L	2	Pgm-2		Pgm-2
SDH	L	1	Sdh		
SOD	L&M	1	Sod		Sod
XDH	L	1			

Table 3. The enzymes examined, the tissues used, the number of loci investigated, the species pair differences (diagnostic loci) found in comparisons between whiting species. Key: L=liver, M=muscle, BASS=*S. bassensis bassensis*, FLIN=*S. bassensis flindersi*, VITT=*S. vittata*.

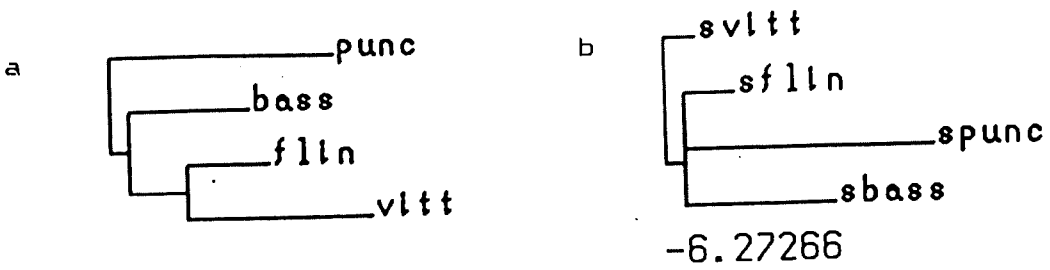


Figure 3: Dendrograms to show the relationships between *S. bassensis bassensis* (bass), *S. bassensis flindersi* (flin), *S. vittata*, (vitt) and *S. punctata* (punc). (a) as determined using isozyme data (CONTML), (b) as determined using isoelectric focusing data (MIX).

comparisons. The occurrence of genuine fixed allelic differences between sympatric species is a very strong indication of the existence of independent gene pools and thus distinct species (Shaklee, 1983). Even one such statistically significant difference between sympatric populations is strong evidence of separate species.

In comparisons between *S. vittata* and *S. bassensis bassensis*, which are sympatric species, fixed differences were found in 14 out of the 43 loci studies (Table 3). These two species are thus distinct despite their superficial similarities. When *S. bassensis flindersi* and *S. vittata* were compared the same number of fixed differences were observed (14/43), although not all of the same loci were involved. These would also be regarded as separate species because, although they are not sympatric, their distributions are so widely separated that the chance of interbreeding in nature would be very remote.

The comparison between *S. bassensis bassensis* and *S. bassensis flindersi* is very interesting. Twelve out of the 43 loci examined showed fixed differences (Table 3). These large differences, we believe, indicate that *S. bassensis bassensis* and *S. bassensis flindersi* are distinct species.

The dendrogram (Figure 3a) which shows the relationships between the species examined was produced using the computer program CONTML in Felsenstein's PHYLIP package (Felsenstein, 1981, 1982). In this analysis *S. punctata* was included as the outgroup. This supports the idea that *S. bassensis bassensis* and *S. bassensis flindersi* are separate species, because the differences between them are almost as great as between either of them and *S. vittata*.

Further evidence supporting this idea is presented in Figure 4. In this case the dendrogram was produced with the same program as above but the input data were the frequencies of alleles in the population of *S. bassensis bassensis* from St. Vincents Gulf and *S. bassensis flindersi*. The *S. bassensis flindersi* data were subdivided and entered separately for eight different localities from Eastern Australia. The dendrogram shows that the differences between *S. bassensis bassensis* and any of the *S. bassensis flindersi* populations is much greater than the differences between any of the *S. bassensis flindersi* populations.

Isoelectric focusing of soluble muscle proteins has also been used to compare *S. bassensis bassensis* and *S. bassensis flindersi*. Distinct differences were again found between them (Figure 5). A dendrogram

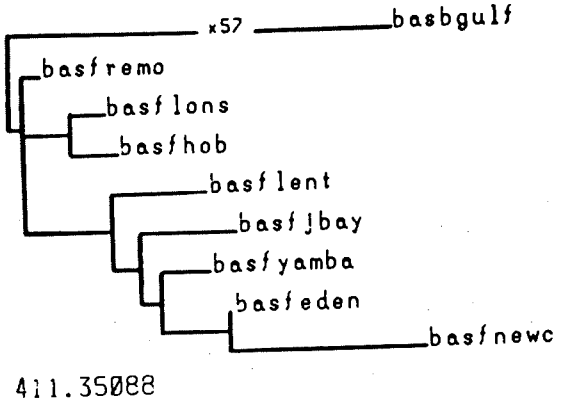


Figure 4: Dendrogram to show the relationships between populations of *S. bassensis bassensis* and *S. bassensis flindersi*.

Key: *S. bassensis bassensis* = basgulf from St Vincent's Gulf: *S. bassensis flindersi* = basfremo from San Remo; = basflons from Pt. Lonsdale; = basfhob from Hobart; = basflent from Lakes Entrance; = basfjbay from Jervis Bay; = basfyamba from Yamba; = basfeden from Eden; = basfnewc from Newcastle.

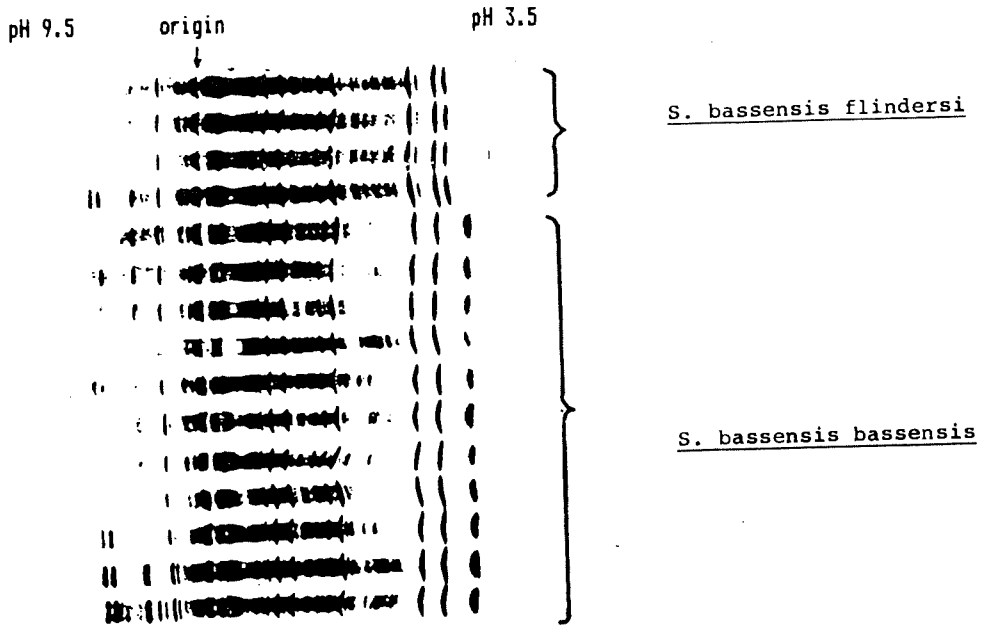


Figure 5: Isoelectric focusing gel of soluble muscle proteins from *S. bassensis flindersi* and *S. bassensis bassensis*.

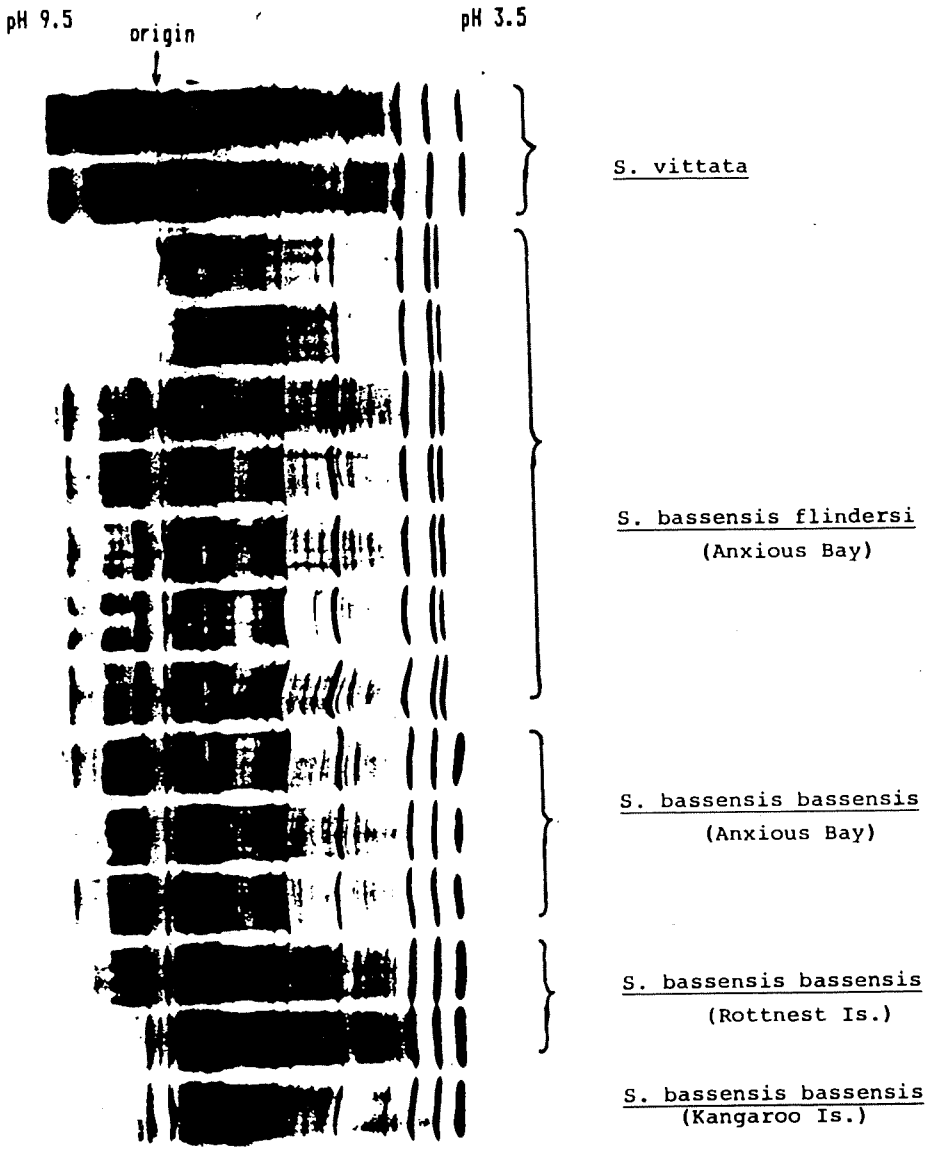


Figure 6: Isoelectric focusing gel of soluble muscle proteins from *S. vittata*, *S. punctata*, *S. bassensis flindersi* from N.S.W., Tasmania and Anxious Bay (S.A.) and *S. bassensis bassensis* from W.A., and Kangaroo Island and Anxious Bay (S.A.).

(Figure 3b) was produced using the MIX program in Felsenstein's PHYLIP package (Felsenstein, 1981, 1982). Although a different arrangement of the species is observed in this dendrogram, the differences between *S. bassensis bassensis* and *S. bassensis flindersi* remain large.

Initially we had not found these two "subspecies" to be sympatric, but eventually a sample was obtained from Anxious Bay on the west coast of South Australia. This sample, of 60 specimens, appeared to consist of three specimens of *S. bassensis bassensis* (the expected form) and 57 *S. bassensis flindersi*, which had not previously been found west of Cape Otway*. These specimens were carefully examined at those loci which had previously been shown to have different alleles in the two sub-species. There was no evidence of introgression between them; the two sub-species remained distinct. Finally isoelectric focusing of soluble muscle proteins (Figure 6) also supported the view that the sample was made up of 57 *S. bassensis flindersi* and three *S. bassensis bassensis*.

Morphometric and Meristic Studies

Morphometric and meristic measurements were made on samples of fish from the two sub-species. These data are summarised below.

Meristics

Summaries of meristic counts are shown in Tables 4-6. From Table 4 it can be seen that no differences were observed between the numbers of first and second dorsal spines and anal spines and rays for the different regions. The number of second dorsal rays varied, however, with the majority of eastern forms (Yamba, Eden and Hobart) having 17 rays whilst the western forms (Spencer Gulf and Mandurah) predominantly having 18 rays. This is further demonstrated in Table 5 which shows the relationship between the number of second dorsal and anal rays for individuals from the various regions. The frequency of second dorsal and anal rays for the eastern forms were predominantly 17 and 19 whilst western forms were predominantly 18 and 19. These results are similar to the observations of McKay (1985).

* *S. bassensis flindersi* has since been obtained from Port Fairy in the Portland area.

	FIRST DORSAL SPINES			SECOND DORSAL SPINES	SECOND DORSAL RAYS				ANAL SPINES	ANAL RAYS			
	10	11	12	1	16	17	18	19	2	17	18	19	20
YAMBA,NSW	-	100	-	100	2	91	6	1	100	-	7	91	2
HOBART,TAS	6	94	1	101	8	89	4	-	101	1	22	77	1
EDEN,NSW	-	100	-	100	5	94	1	-	100	-	3	95	2
SPENCERS GULF,SA	3	96	-	99	-	8	81	10	99	-	7	90	2
MANDURAH,WA	1	93	2	96	-	4	82	10	96	-	7	77	12

Table 4. Summary of the frequency distributions of school whiting dorsal and anal fin spines and rays by geographic area.

SECOND DORSAL RAYS	16	16	17	18	16	17	18	19	17	18	19	N
ANAL RAYS	17	18	18	18	19	19	19	19	20	20	20	
YAMBA	-	1	6	-	1	84	5	1	1	1	-	100
HOBART	1	2	20	-	5	69	3	-	-	1	-	101
EDEN	-	2	1	-	3	91	1	-	2	-	-	100
MANDURAH	-	-	3	4	-	1	71	7	-	7	3	96
SPENCER GULF	-	-	5	2	-	3	78	9	-	1	1	99

Table 5. Summary of frequency distributions of school whiting second dorsal and anal fin rays by geographic area.

AREA	LATERAL LINE SCALES									ANAL SCALES		DORSAL SCALES	
	65	66	67	68	69	70	71	72	73	9	10	4	5
YAMBA	4	4	5	4	10	12	16	-	1	1	50	-	56
MANDURAH	-	-	-	-	1	2	-	3	1	-	9	-	22
HOBART	-	-	-	2	16	22	7	5	-	30	43	1	78
EDEN	-	-	-	-	2	27	38	25	6	16	82	-	99
SPENCER GULF	-	-	-	-	-	-	-	-	-	1	10	-	36

Table 6. Summary of frequency distributions of school whiting lateral line, anal and dorsal scale counts by geographic area.

Due to the poor condition of many specimens, the results of dorsal, anal and lateral line scale counts were inconclusive (Table 6). In many instances, counts could only be made by including adjacent scale rows which lead to considerable variation possibly due to counting error. It is evident, however, that the majority of individuals from the five regions had ten scale rows between the origin of the anal fin and the lateral line and five scale rows between the first dorsal fin origin and the lateral line. Lateral line scales were particularly difficult to count and in many instances scales were nonexistent in the caudal flexure region. In respect of the Spencer Gulf sample, no lateral line scale counts were possible. It is likely, however, that the number of lateral line scales were in the range of 69 to 72 scales for the four regions from which counts were possible.

Morphometrics

Effects of transformations on data

Descriptive statistics for RATIO, LGRATIO and ALLOM are given in Appendix 8 (Tables 8.1 - 8.3). Transforming the data radically decreased the values of means and variances for LGRATIO and ALLOM whilst values for RATIO were only moderately affected and are directly comparable with the results presented by McKay (1985). Particularly noteworthy is the variable HEHL (HW in HL) which shows significant differences (95% confidence limits) between the eastern and western forms for the three transformations.

The effects of the various transformations on the normality of variables is given in Appendix 9 (Tables 9.1 - 9.3). For RATIO, seven variables showed significant skewness and eight variables showed significant kurtosis. LGRATIO demonstrated similar results with seven and nine variables showing significant skewness and kurtosis, respectively. Transforming raw data using the ALLOM method increased non-normality (particularly SNHL) with nine and 13 shape variates being significantly skewed and kurtose, respectively. Regardless of the type of transformation, shape variates of individuals from Hobart and Spencer Gulf were virtually all normally distributed.

Efficacy of size removal

Appropriate statistics for the simple linear regression of size on shape are given in the Appendix 10 (Tables 10.1 - 10.3). Only ALLOM showed no significant

relationship of shape with the size variate SL. For RATIO, three or more shape variates were significantly associated with SL in each region and the average r^2 was 0.115. Similar results were obtained for LGRATIO with 24 of the 40 possible variables being significantly associated with SL with the average r^2 being 0.116. For ALLOM, no shape variates were significantly associated with SL and the mean r^2 was 0.00017.

Discriminant analysis of covariance structure

The pooled within-groups correlations between canonical discriminant functions and discriminating variables for the three transformations are shown in Table 7. Whilst the sequence of variable entry, magnitudes of the coefficients and partitioning of variance onto discriminant axes varied for the three transformations, the main discriminating variable was HEHL in all cases.

The general pattern of centroid positions in discriminant space was similar for all types of transformation (Figure 7). The overlap of 90% isodensity circles between the groups Yamba, Hobart and Eden suggests that the morphology of these individuals is very similar. Conversely, the lack of overlap of confidence limits between the above groups and Spencer Gulf and Mandurah suggests these groups have different phenotypes.

Clustering the correlations between the centroids (UPGMC) for the four canonical discriminant functions for the five areas (Figure 7) indicated that, overall, Hobart, Eden and Yamba were most similar to each other. Furthermore, Spencer Gulf and Mandurah formed another group that was similar but not as closely related to each other as the previous group. Differences in statistical association of these groups does not lead to differences in biological interpretation based upon each of these data types.

In summary, the various transformations used to produce shape variates affected normality, correlations and covariances, but this did not lead to any differences in biological interpretation. Whilst the underlying assumptions of discriminant analysis of multi-variate normality and equality of variance-covariance matrices within each group were not strictly adhered to, Nie et al. (1975) suggested that this technique is very robust and that these assumptions need not be strictly adhered to. While all transformations were efficient in removing some size information, ALLOM performed best, resulting in a discriminant analysis solution whereby 89.3% of cases were correctly classified to their

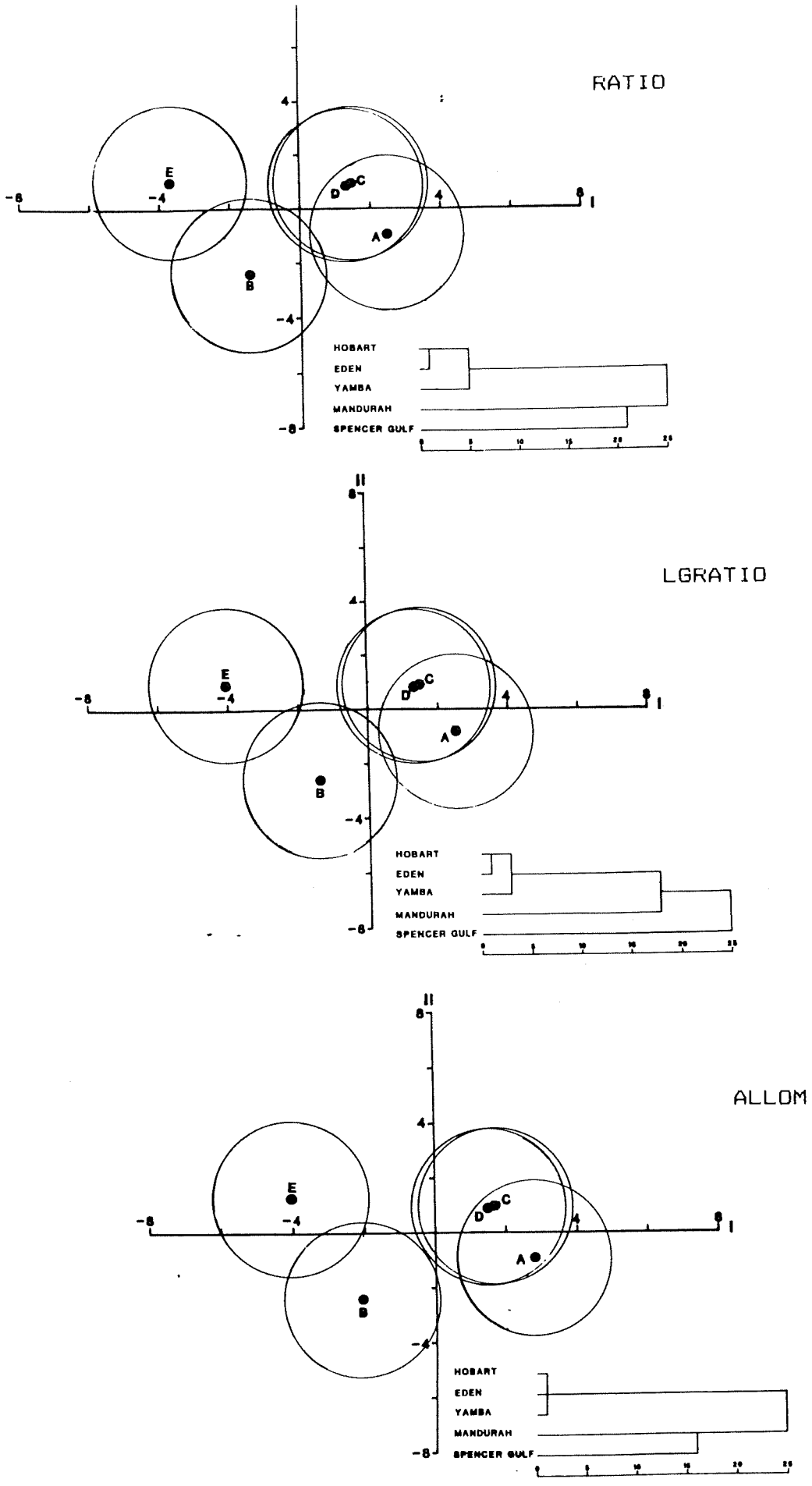


Figure 7: Group centroids for RATIO, LGRATIO and ALLOM shape variates positioned in discriminant space for functions I and II. Isodensity circles contain 90% of group cases (Note: A, Yamba; B, Mandurah; C, Hobart; D, Eden and E, Spencer Gulf. Clustering of group centroids by UPGMC for all possible functions for each data type.

particular group. RATIO and LGRATIO both produced a correct classification rate of 83.5%.

The use of discriminant analysis in ichthyological numerical taxonomy could lead to erroneous biological interpretations where the morphological measurement cannot be made with consistent accuracy. For instance, variability within measurements of soft body parts such as snout to anal fin origin could be due to either biological variability or to measurement error. Discriminant analysis based solely on measurements of soft parts could therefore be hazardous. The main discriminating variable in this study (HEHL) is a bony structure and the results are therefore considered indicative of phenotypic variation.

Thus both the electrophoretic and morphometric data strongly support the view that *S. bassensis bassensis* and *S. bassensis flindersi* are actually distinct species, and we will shortly describe them as such.

The distributions of these two species is shown in Figure 8. When compared to the distributions described by McKay it is apparent that *S. bassensis flindersi* is distributed much further westwards than previously reported; its distribution extends westwards at least to Anxious Bay in South Australia. *Sillago bassensis bassensis* has so far been found to extend eastwards only as far as Kangaroo Island and St. Vincents Gulf. However no sampling has been carried out between Portland and Kangaroo Island so it is possible that it actually extends further eastwards. All of the specimens collected east of Portland have been *S. bassensis flindersi*.

Sillago bassensis flindersi.

We have examined the population structure of *S. bassensis flindersi* throughout its range with a view to obtaining information that will assist in managing what appears to be a growing fishery. We have used starch gel electrophoresis to study seven polymorphic enzyme loci (Pgd, Aat-2, and Adh from liver and Mpi, Gpi-1, Gpi-2 and Gpi-3 from muscle) in fish from 21 localities. These seven loci out of 19 suspected polymorphic loci (see Table 3.4, Appendix 3) proved to be the most reliable for ease of genetic interpretation. Refer to Appendix 6 for a description of the enzyme banding patterns for these polymorphic loci. Only at the Mpi and Pgd loci were the frequencies of the most common allele less than 0.90. The allele frequencies at each of these loci and the numbers of specimens used in each population are given in Table 7.1, Appendix 7.

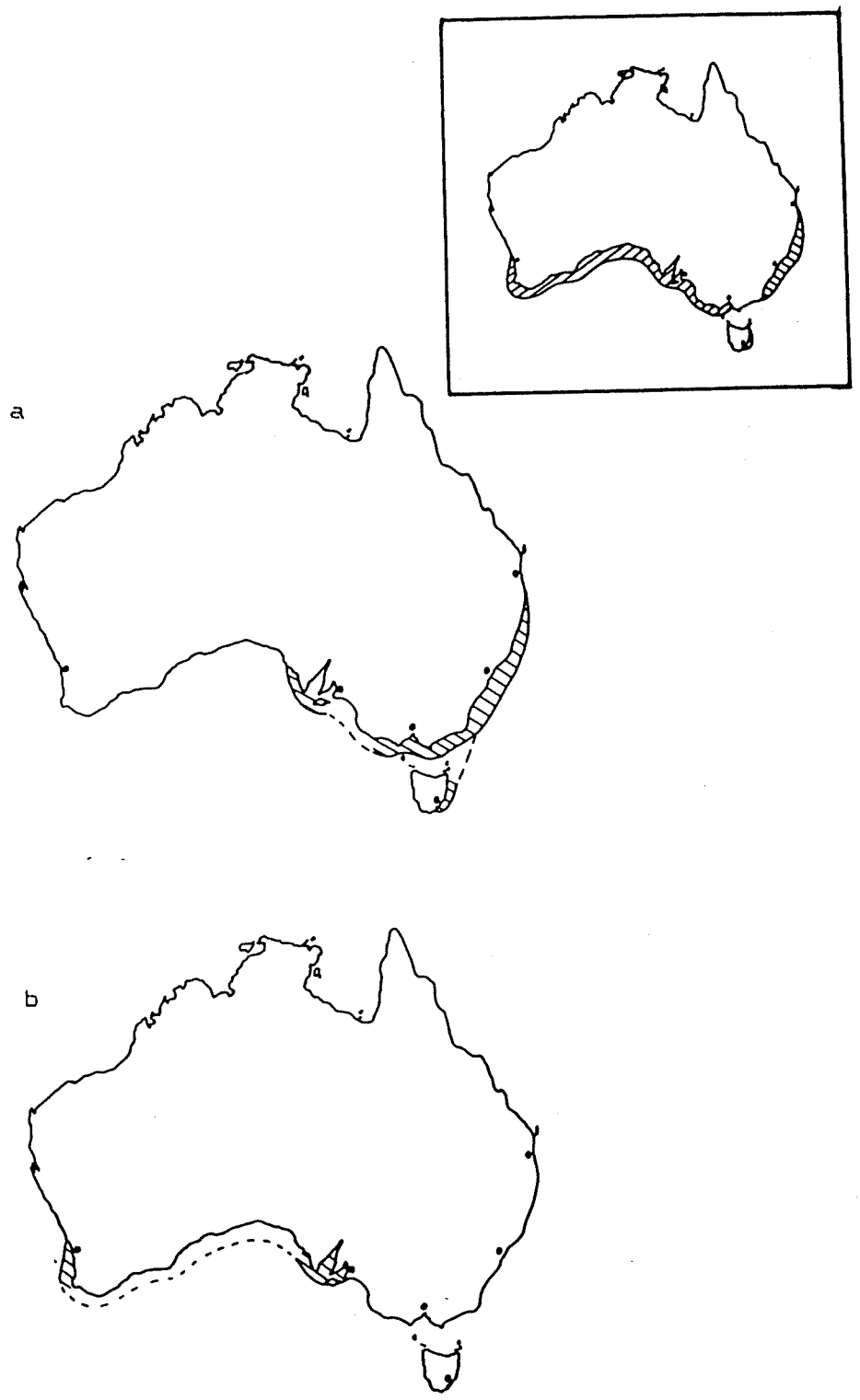


Figure 8: Maps to show the distribution of (a) *S. bassensis flindersi* and (b) *S. bassensis bassensis*. Inset shows distribution described by McKay (1985).

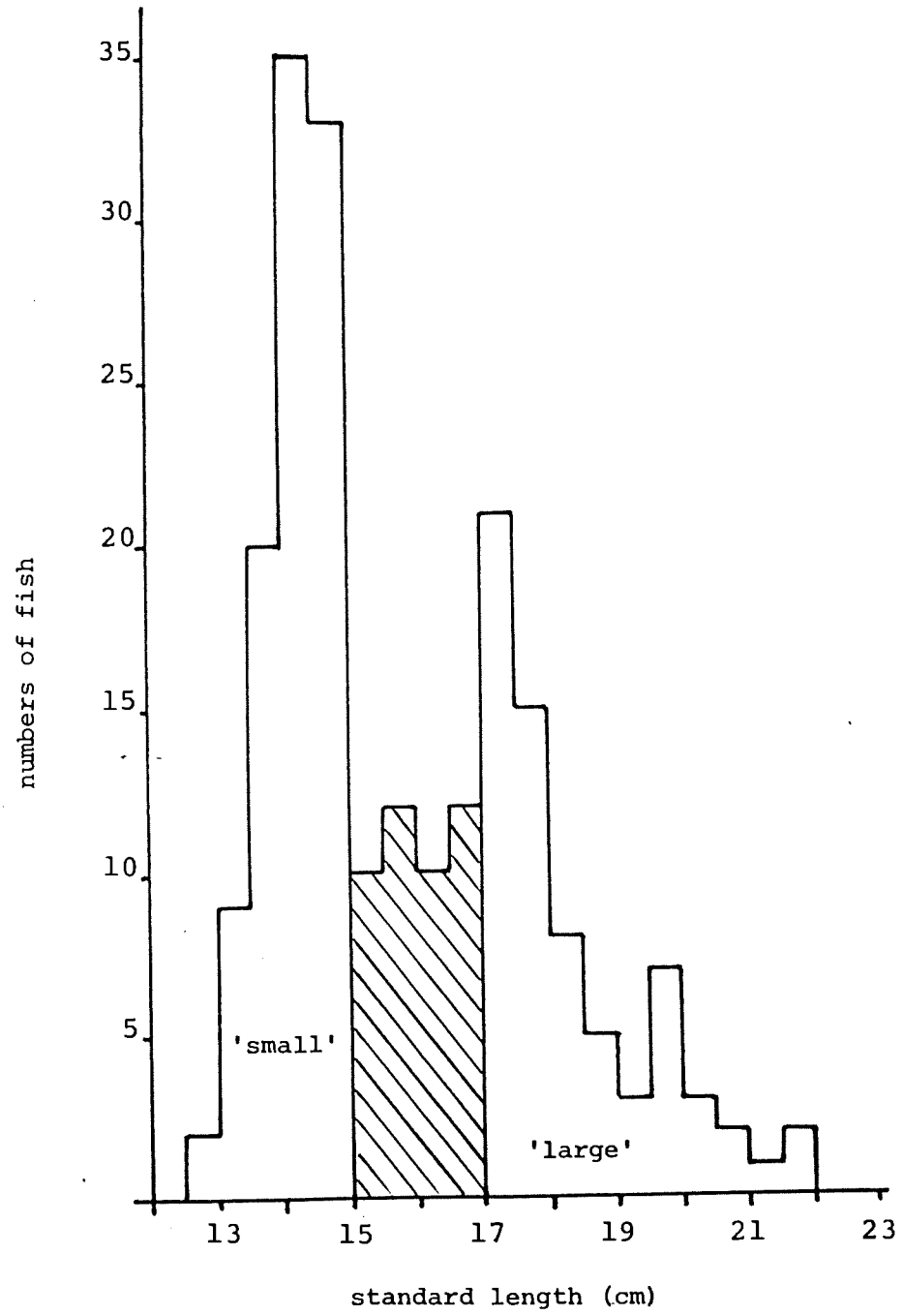


Figure 9: Size frequency distribution of *S. bassensis flindersi* from Yamba 1, to show the sizes of individuals in the 'small' and 'large' subgroups.

At some localities multiple samples were taken. Samples were taken from Yamba on three occasions (7/6/84, 22/5/86 and 23/5/86); the sample of 7/6/84 was larger than the others (200) and was sub-divided into two groups, one "large" the other "small". This division was carried out in the following way. First, all of the fish were measured (SL) and the size frequency distribution plotted. The fish from each end of the distribution were designated "small" or "large" and those from an overlap region of 2 cm (SL) were not included in subsequent analyses (Figure 9). Two samples were taken from the Camden Heads area on the same day; three samples were taken from Forster (1/10/84, 5/6/85 and 20/5/86); two samples were taken from the Coffs Harbour area (2/4/85 and 21/5/86) and two samples were taken from the Cape Patton area on the same day (30/9/85).

The allele frequency data collected from these samples were used to construct dendrograms to show the genetic relationships between the populations. Again we used Felsenstein's (1981, 1982) CONTML and FITCH programs. The dendrograms of highest likelihood are shown in Figures 10 and 11.

Examination of the groupings in the dendrograms shows that the populations are not clustered according to geographic proximity. For example, in the CONTML plot (Figure 10) one grouping includes Eden and Yamba, another includes Camden Heads (N.S.W.), Apollo Bay and Cape Patton (Vic.), sample 2 whereas Cape Patton, sample 1 is clustered with Jervis Bay (N.S.W.), and yet another cluster includes Port Fairy (Vic.), Anxious Bay (S.A.) and North Solitary Is. (N.S.W.). The FITCH plot (Figure 11) shows the populations grouped somewhat differently, but once again the groupings are not as expected on geographical grounds. We place more weight on the groupings as shown using CONTML because this is the preferred program for handling gene frequency data (see discussion in Appendix 5).

G-tests, which provide a simple, yet powerful test for distinguishing between populations, were performed on all possible pairs of populations. The detailed results are found in Table 7.2, Appendix 7. Out of the 437 comparisons made, the differences between 164 of them were significant.

A summary of these results is seen in Figure 12. Examination of this figure reveals that there is a major discontinuity between the populations in the region between Forster and Coffs Harbour.

The loci which contribute most to the differences between the populations are Mpi and Pgd. The geographic variation at these loci is shown in Figures

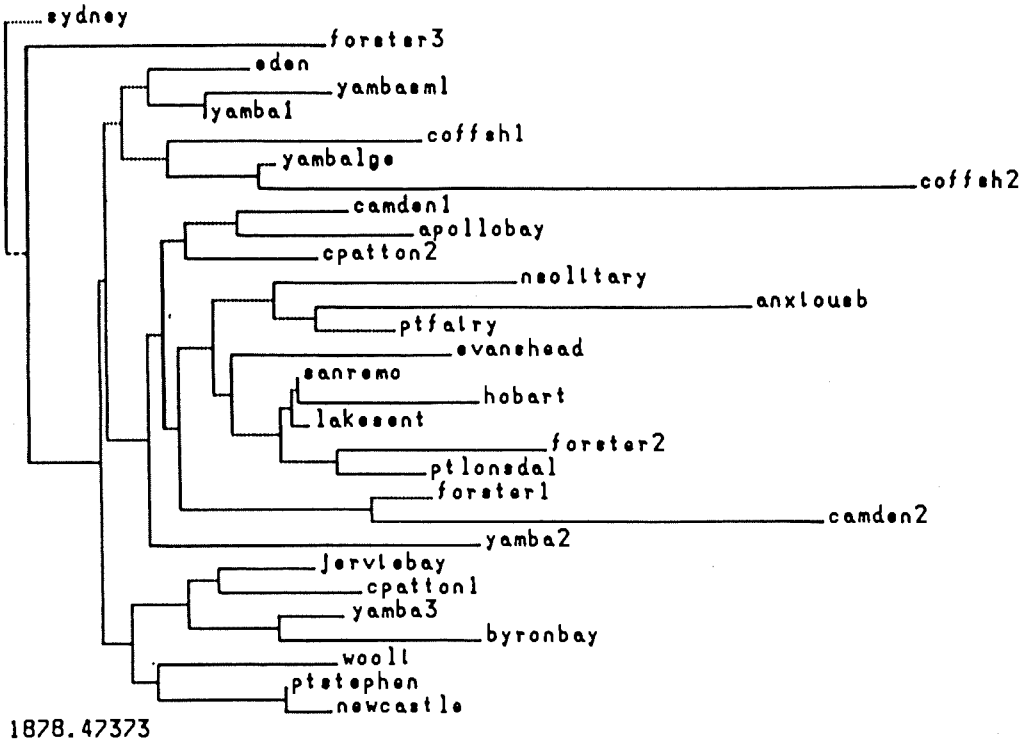


Figure 10: Dendrogram to show relationships between samples of *S. bassensis flindersi* from different localities (CONTML plot).

Key: byronbay = Byron Bay; evanshead = Evans Head; yambaltot = Yamba (7/6/84) total; yambalge = Yamba (7/6/84) large; yambalsml = Yamba (7/6/84) small; yamba2 = Yamba (22/5/86); yamba3 = Yamba (23/5/86); wooli = Wooli; nthsolit = Nth Solitary Island; coffsh1 = Coffs Harbour (2/4/85); coffsh2 = Coffs Harbour (21/5/86); camden1 = Camden (2/10/85); camden2 = Sth Camden Heads (2/10/85); forster1 = Forster (1/10/85); forster2 = Forster (5/6/85); forster3 = Forster (20/5/86); ptstephen = Port Stephens; newcastle = Newcastle; sydney = Sydney; jervisb = Jervis Bay; eden = Eden; lentrance = Lakes Entrance; sanremo = San Remo; ptlonsd = Pt Lonsdale; cpatton1 = Cape Patton (area 1); cpatton2 = Cape Patton (area 2); apollob = Apollo Bay; ptfairy = Port Fairy; hobart = Hobart; anxiousb = Anxious Bay

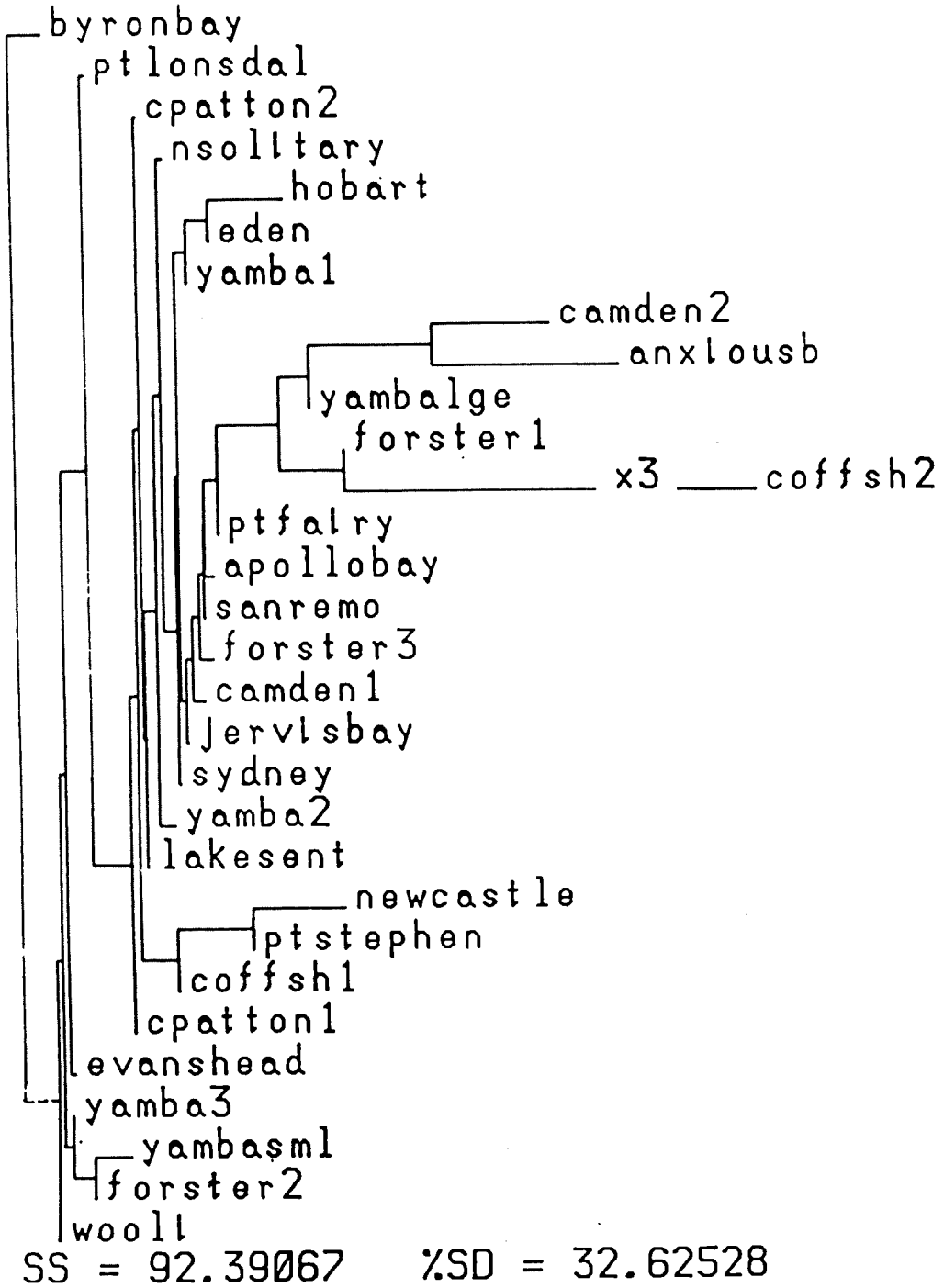


Figure 11: Dendrogram to show relationships between samples of *S. bassensis flindersi* from different localities (FITCH plot). Key as for Figure 10.

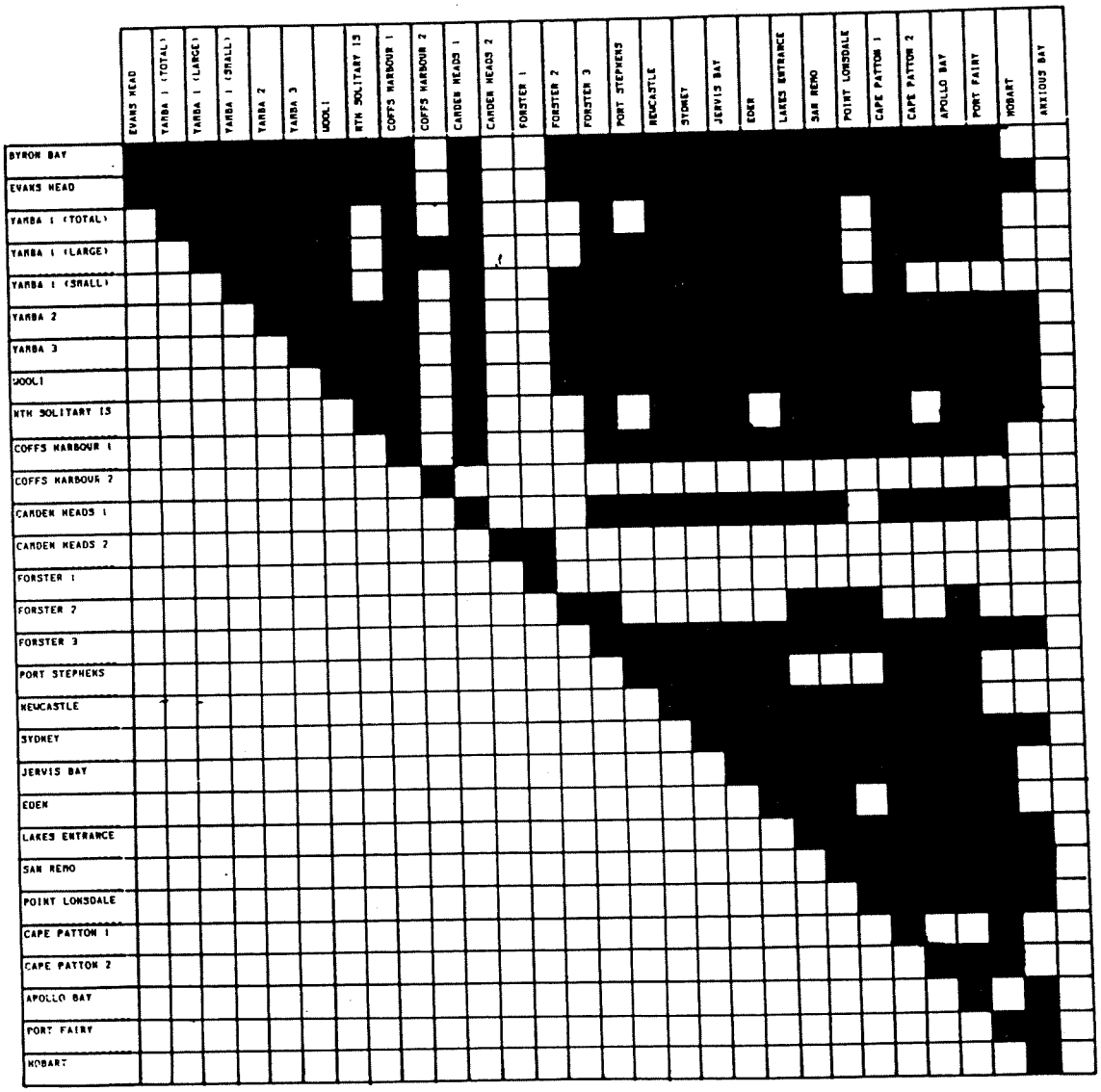


Figure 12: Summary of the results of G-tests on gene frequency data, for all combinations of samples of *S. bassensis flindersi*. Only the top half of the matrix is completed.

□ = significant G-test, ■ = nonsignificant G-test.

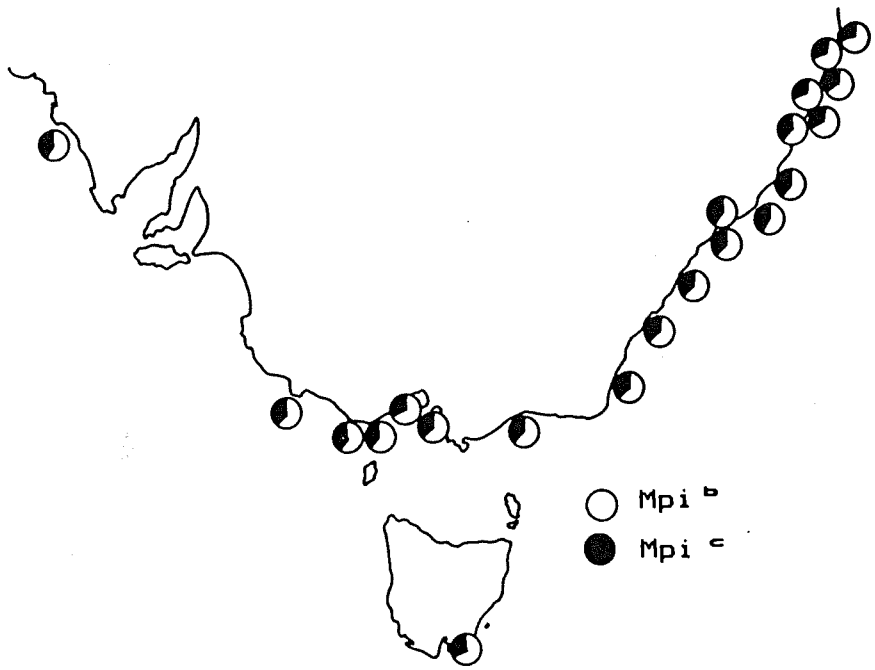


Figure 13: Frequencies of Mpiᵇ and Mpiᶜ throughout the distributional range of *S. bassensis flindersi*.

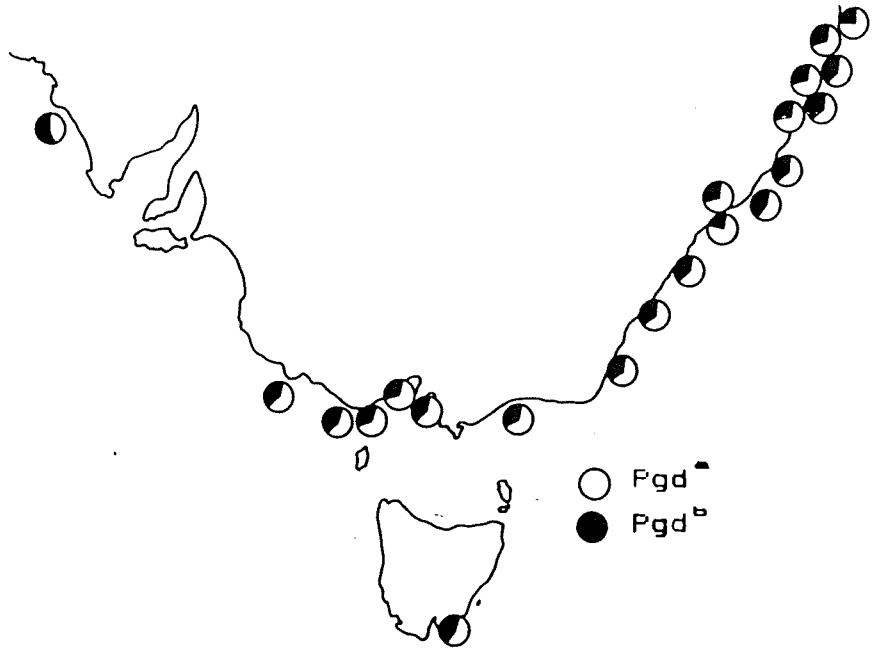


Figure 14: Frequencies of Pgdᵃ and Pgdᵇ throughout the distributional range of *S. bassensis flindersi*.

13 and 14. There is no evidence of clinal variation: the differences are haphazard in their arrangement.

Sillago bassensis flindersi is a small fish which has a high level of natural mortality (D. Smith, pers. comm.). It was initially suggested to us that because of this mortality and its suspected low mobility, this species may consist of several localised populations with some degree of isolation. The apparent haphazard relationships between fish from different localities suggest that this may be the case. This hypothesis, however needs closer examination.

There are three alternatives that must be considered when we interpret our data:

(1) there are many small populations,

(2) practically the whole area is occupied by one population, or

(3) there are only a few populations of *S. bassensis flindersi*.

Now let us consider these alternatives. It is apparent that we have shown that significant geographic differentiation occurs between some populations. It has also been observed that the distribution of *S. bassensis flindersi* is very patchy in N.S.W. (Smith, 1985) and it is difficult to obtain any samples at all between Forster and Eden. It is unfortunate that there are no tagging data available, because these would test the model based on the genetic data. Thus, if tagging indicated that the fish made only small movements, then such an observation would be consistent with the idea that this species is made up of multiple, small populations. However it seems unlikely that the oceanographic conditions on the east coast would be conducive to the development of many 'isolated' populations in a species that lives, and is assumed to spawn, at sea. In this case the eggs and larvae could be carried considerable distances between spawning and the time when the larvae "settle out". The length of larvae life is thought to be of the order of one month (A. Miskiewicz, pers. comm.).

Perhaps the fish from practically the whole area studied actually form one large population. A possible model to explain the observed genetic relationships between our samples could be that:

(1) relatively small groups of fish reach the spawning area to reproduce,

(2) chance genetic differences between these groups result in offspring groupings from each spawning area that differ genetically,

(3) this results in a shifting pattern of geographic differentiation in a haphazard manner.

This model could be eliminated if:

(1) the natural history of the fish is against

it, or

(2) samples of different age classes from the same locality are more similar to each other than to those from other areas.

Little is known about the life history of the fish; it is not known where the spawning areas are located, although they are thought to be on the continental shelf. The length of larval life is not known but it is suspected to be about one month. It seems, then, unlikely that this alternative could be eliminated on these grounds.

A genetic comparison was made between 'large' and 'small' specimens from Yamba. This is the only locality from which we have, to date, been able to obtain sufficient fish at the one time to carry out such a comparison. The G-test, which was carried out to compare Yamba 'large' with Yamba 'small' fish, was not significant (G=36.7305, p=0.0612, see Table 7.2 Appendix 7). However, the frequencies of Mpi and Pgd in the 'small' and 'large' fish were quite different (Figure 15), indicating the need for future work. These were the only two loci where the frequency of the most common allele was less than 0.90 and when the G-test was repeated using only these data the result was a significant difference (G=20.2013, p=0.002).

While the results from one locality could arise by chance, these results may be used to frame hypotheses at other localities. This we did for Forster, Coffs Harbour and Camden Heads; we examined further samples from these localities. The null hypothesis tested was that the genetic composition of samples from the same area will remain the same with time. Three samples were compared from Forster and G-tests indicated that there were significant differences between all of them (Table 7.2, Appendix 7). The two samples from Coffs Harbour, which were taken about a year apart were significantly different. We also compared the two samples from Camden Heads. These samples were taken only one day apart from a locality slightly north of the previous one; they both differed significantly. Similarly the two samples taken from Cape Patton were significantly different. The frequencies of Mpi and Pgd in all these samples are shown in Figure 15.

G-tests were carried out to compare the Pgd and Mpi frequencies for different samples at different sites. The results of these tests are given in Tables 7 and 8. At the Pgd locus significant results were obtained for Yamba "large" versus Yamba "small" and Yamba "large" versus Yamba 3. The same comparisons gave a significant G-test at the Mpi locus. In addition, for Mpi, the comparisons between the two Coffs Harbour samples, the two samples from Camden Heads and Forster

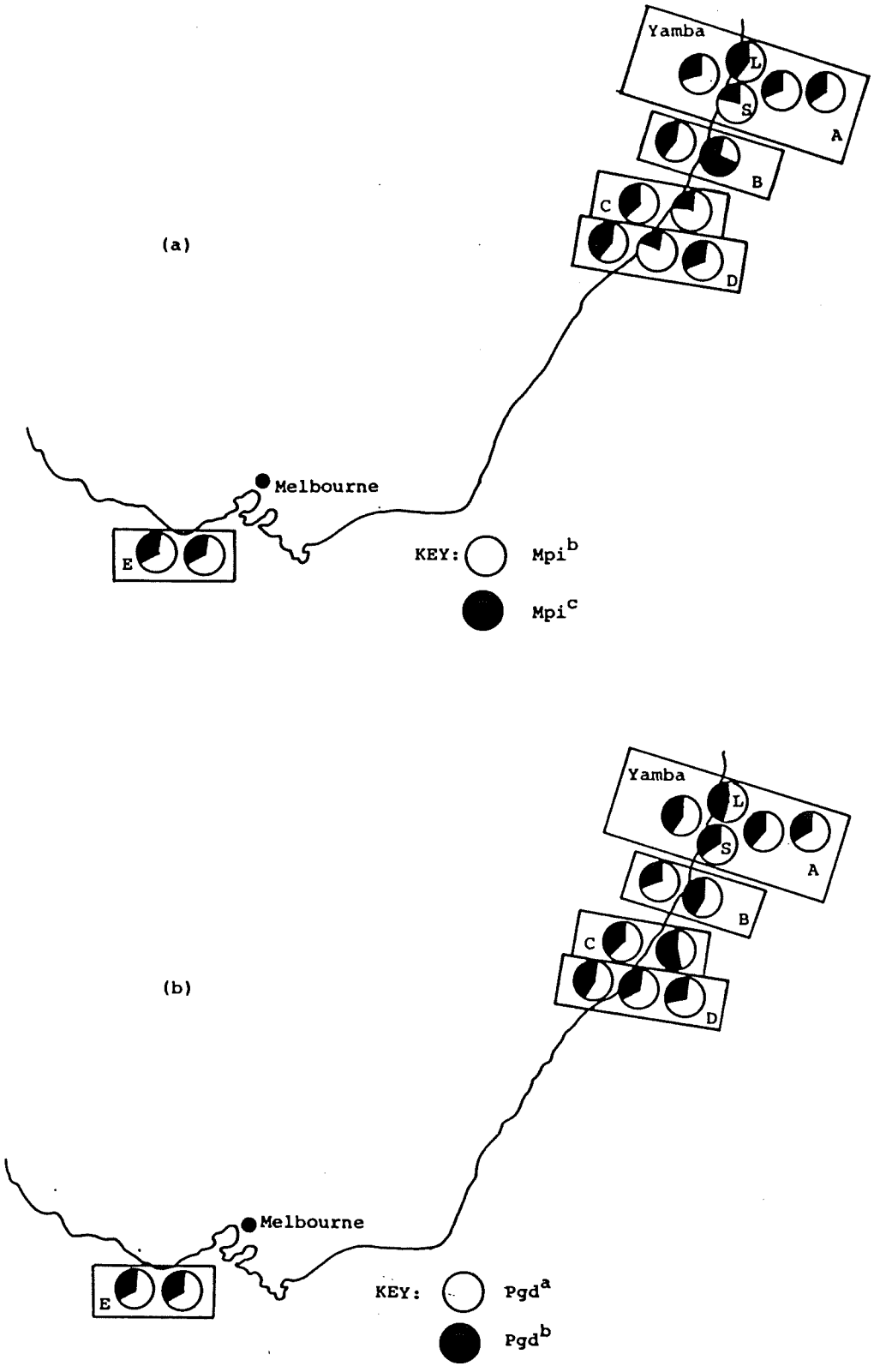


Figure 15: Frequencies of (a) Mpi^b and Mpi^c and (b) Pgd^a and Pgd^b in samples of *S. bassensis flindersi* from localities at which more than one sample was taken.

Key: L = 'large', S = 'small',
A = Yamba, B = Coffs Harbour,
C = Camden Heads, D = Forster,
E = Cape Patton.
1, 2 and 3 indicate samples taken at different times.

otu 1	vs	otu 2	g stat	d of f	prob.
Yambaltot		yamballge	2.435675	1	.1185
		yambalsml	2.25425	1	.1333
		yamba2	5.51440e-3	1	.9410
		yamba3	2.12054	1	.1454
yamballge		yambalsml	6.53494	1	.0106*
		yamba2	.931985	1	.3344
		yamba3	5.75696	1	.0164*
yambalsml		yamba2	1.05898	1	.3035
		yamba3	5.73753e-2	1	.8108
yamba2		yamba3	1.24703	1	.2642
coffs1		coffs2	3.39485	1	.0654
camden1		camden2	2.05715	1	.1515
forster1		forster2	1.41353	1	.2345
		forster3	7.00490e-3	1	.9335
forster2		forster3	.881059	1	.3480
cpatton1		cpatton2	3.54180e-3	1	.9527

Table 7. Comparisons between samples (G-tests) of *S. bassensis flindersi* at the Pgd locus. * indicates a significant result.

otu 1	vs	otu 2	g stat	d of f	prob.
yambaltot		yamballge	2.66995	1	.1023
		yambalsml	7.52029	1	.0061*
		yamba2	.25679	1	.6124
		yamba3	1.37011	1	.2418
yamballge		yambalsml	13.3518	1	.0003*
		yamba2	2.3558	1	.1248
		yamba3	5.0954	1	.0240*
yambalsml		yamba2	1.6473	1	.1994
		yamba3	1.0077	1	.3155
yamba2		yamba3	.153493	1	.6953
coffsh1		coffsh2	17.2947	1	0.000*
camden1		camden2	4.41882	1	0.356*
forster1		forster2	13.4466	1	.0002*
		forster3	2.01203	1	.1561
forster2		forster3	1.89978	1	.1681
cpatton1		cpatton2	1.58858e-3	1	.9684

Table 8. Comparisons between samples (G-tests) of *S. bassensis flindersi* at the Mpi locus. * indicates a significant result.

1 and Forster 2, yielded significant results. Thus repeated sampling in the same areas does not always give the same result. The variability observed within these sites is as great as the variability observed over the whole range of the species.

That plankton is patchy in its distribution is well known (Fasham, 1978). Recently, oceanographers have shown that such heterogeneity can be forced by physical factors in the ocean (Denman and Powell, 1984; Haury *et al.*, 1983). Mackas *et al.* (1985) show a striking similarity between biological and physical satellite images obtained from the same area at the same time; the same swirls, streaks and eddies are visible in both. It is apparent, then, that plankton patchiness is strongly influenced by hydrodynamic processes.

We believe that the lack of consistency which we observed between samples of *S. bassensis flindersi* is due to patchy recruitment to the different areas. Such patchiness in recruitment is easily explained if the hydrodynamic processes in the ocean produce water pockets in which larvae are trapped until they "settle out". If many such pockets exist then the larvae in each could well be the result of the spawnings of relatively few individuals.

When all these aspects are taken together we are unable to eliminate the possibility that fish from practically the whole area belong to the one population. It is apparent, however, that given the large distances involved many groups of these fish would be isolated by distance.

However, in Figure 12 we can see that there is a discontinuity in the region between Coffs Harbour and Forster. It is likely that this represents a true discontinunity, because in oceanographic terms this area is complex.

In the area between Smoky Cape (just south of Coffs Harbour) and Sugarloaf Point (just south of Forster) the East Australian Current (EAC) commonly turns eastwards from the coast (Godfrey *et al*, 1980). In the summer Sugarloaf Point is the most common separation point but in winter separation occurs further north. Cresswell *et al.* (1983) report many "fronts" in the ocean in this area and Rochford (1975) found upwelling to occur near Camden Heads. As well as this, examination of NOAA satellite images reveals many small water bodies between the coast and the EAC. Thus, this is a complex area oceanographically and is likely to provide barriers to dispersal.

Such barriers could act as a moving boundary between two sub-populations of *S. bassensis flindersi* on the east coast of the continent. In general terms these sub-populations could be regarded as occurring:
(i) from about Forster north;
(ii) south of Forster
The boundaries between the sub-populations should not be regarded as fixed; there is probably yearly and seasonal variation.

The idea of two sub-populations of *S. bassensis flindersi* on the east coast is consistent with the findings of Smith (1985) who studied the Gonadosomatic Index (GSI) of fish from Eden and Yamba. He found that the GSI was at a maximum in the winter in the from Yamba, and in the summer in those from Eden. Further studies on the GSI, and histological examination of gonad development in fish from localities between Yamba and Eden should be undertaken to determine whether the time of spawning is clinal or whether it too shows perturbation on the mid-north coast.

The extent of the two proposed sub-populations is outlined in Figure 16. The southern sub-population extends westwards to Portland. It is apparent that if the scheme illustrated in Figure 16 is correct, there is great potential for mixing between populations.

Often when interbreeding occurs between populations of different genetic composition, significant heterozygote deficiency occurs. Examination of the genotype frequency data for Mpi (Table 9) reveals that 13/30 of the samples were out of Hardy-Weinberg frequency (H-W) but only two of these displayed heterozygote deficiency. In the case of Pgd (Table 10) eight samples were out of H-W equilibrium and only three of these showed heterozygote deficit.

Thus, there is little evidence from this source that mixing of populations is occuring, but this does not mean that there is no gene flow between populations, the oceanographic processes would almost certainly ensure that gene flow does occur.

With regard to the Anxious Bay sample, it is significantly different from all other samples (Figure 12 and Table 7.2, Appendix 7), and even though we have no samples between there and the Portland area, it is unlikely to belong to an eastern stock. The large distance between Anxious Bay and the eastern localities would mean that the fish would almost certainly be isolated by distance. We have just received a sample, which we believe to be *S. bassensis flindersi*, from the eastern end of Kangaroo Island. The future analysis of this sample will prove interesting.

POPULATION	(PGD) P		GENOTYPE AA	AB	BB	G-ST	PROB.
BYRON BAY	0.743	OBS	21	13	3	0.248	0.783
		EXP	20.4	14.1	2.4		
EVANS HEAD	0.695	OBS	21	15	5	0.771	0.533
		EXP	19.8	17.4	3.8		
YAMBA 1 (TOTAL)	0.602	OBS	71	76	34	2.750	0.062
		EXP	65.6	86.7	28.7		
YAMBA 1 (LARGE)	0.523	OBS	19	30	16	0.364	0.700
		EXP	17.8	32.4	14.8		
YAMBA 1 (SMALL)	0.671	OBS	36	34	9	0.050	0.951
		EXP	35.6	34.9	8.5		
YAMBA 2	0.597	OBS	11	15	5	0.001	0.999
		EXP	11	14.9	5		
YAMBA 3	0.686	OBS	22	15	6	1.513	0.219
		EXP	20.2	18.5	4.2		
WOOLI	0.702	OBS	45	35	9	0.305	0.742
		EXP	43.9	37.2	7.9		
NTH SOLITARY IS	0.601	OBS	35	37	17	1.567	0.207
		EXP	32.1	42.7	14.2		
COFFS HARBOUR 1	0.698	OBS	21	18	4	0.203	0.818
		EXP	20.9	18.1	3.9		
COFFS HARBOUR 2	0.561	OBS	14	18	9	0.484	0.622
		EXP	12.9	20.2	7.9		
CAMDEN HEADS 1	0.605	OBS	38	39	18	1.879	0.151
		EXP	34.8	45.4	14.8		
CAMDEN HEADS 2	0.469	OBS	6	3	7	6.690	0.002 *
		EXP	3.5	8.0	4.5		
FORSTER 1	0.564	OBS	16	21	10	0.414	0.667
		EXP	14.9	23.1	8.9		
FORSTER 2	0.647	OBS	22	22	7	0.047	0.954
		EXP	21.3	23.3	6.4		
FORSTER 3	0.571	OBS	9	14	5	0.031	0.969
		EXP	9.1	13.7	5.1		
PORT STEPHENS	0.669	OBS	33	25	10	1.670	0.187
		EXP	30.5	30.1	7.5		
NEWCASTLE	0.755	OBS	34	15	6	3.506	0.029 *
		EXP	31.4	20.3	3.3		
SYDNEY	0.611	OBS	23	42	7	3.851	0.021 *
		EXP	26.9	34.2	10.9		
JERVIS BAY	0.617	OBS	39	38	17	1.937	0.142
		EXP	35.8	44.4	13.8		
EDEN	0.612	OBS	42	31	21	8.716	0.000 *
		EXP	35.2	44.6	14.2		
LAKES ENTRANCE	0.651	OBS	45	35	16	3.674	0.025 *
		EXP	40.7	43.6	11.7		
SAN REMO	0.589	OBS	36	34	20	4.368	0.013 *
		EXP	31.2	43.6	15.2		
POINT LONSDALE	0.657	OBS	22	23	6	0.000	1.000
		EXP	22	23	6		
CAPE PATTON 1	0.656	OBS	34	50	6	4.944	0.008 *
		EXP	38.7	40.7	10.6		
CAPE PATTON 2	0.653	OBS	34	47	7	2.932	0.052
		EXP	37.5	39.9	10.6		
APOLLO BAY	0.584	OBS	28	34	15	0.666	0.524
		EXP	26.3	37.4	13.3		
PORT FAIRY	0.567	OBS	32	46	19	0.112	0.894
		EXP	31.2	47.6	18.2		
HOBART	0.547	OBS	34	33	27	9.160	0.000 *
		EXP	28.7	47.6	19.7		
ANXIOUS BAY	0.427	OBS	8	25	15	0.198	0.822
		EXP	8.7	23.5	15.8		

Table 9. Pgd gene frequencies (p), observed (obs) and expected (exp) genotype frequences, G-statistic and probabilities for goodness of fit to the Hardy-Weinberg distribution for samples of *S. bassensis flindersi*. * indicates significant deviation from the H-W distribution.

POPULATION	(MPI) Q		GENOTYPE BB	BC	CC	G-ST	PROB.
BYRON BAY	0.713	OBS	18	21	1	3.669	0.025 *
		EXP	20.3	16.4	3.3		
EVANS HEAD	0.700	OBS	23	17	5	0.445	0.647
		EXP	22.1	18.9	4.0		
YAMBA 1 (TOTAL)	0.671	OBS	92	55	31	15.61	0.000 *
		EXP	80.1	78.6	19.3		
YAMBA 1 (LARGE)	0.591	OBS	27	24	15	4.063	0.017 *
		EXP	23.1	31.9	11.0		
YAMBA 1 (SMALL)	0.784	OBS	59	20	9	8.409	0.000 *
		EXP	54.1	29.8	4.1		
YAMBA 2	0.703	OBS	17	11	4	0.965	0.617
		EXP	15.8	13.4	2.8		
YAMBA 3	0.731	OBS	28	20	4	0.026	0.975
		EXP	27.8	20.4	3.8		
WOOLI	0.705	OBS	46	42	7	0.399	0.677
		EXP	47.2	39.5	8.3		
NTH SOLITARY IS	0.691	OBS	53	28	16	9.852	0.000 *
		EXP	46.3	41.4	9.3		
COFFS HARBOUR 1	0.616	OBS	19	15	9	3.167	0.041 *
		EXP	16.3	20.3	6.3		
COFFS HARBOUR 2	0.295	OBS	8	7	24	12.21	0.000 *
		EXP	3.4	16.2	19.4		
CAMDEN HEADS 1	0.609	OBS	32	42	13	0.016	0.985
		EXP	32.3	41.4	13.3		
CAMDEN HEADS 2	0.750	OBS	19	13	2	0.013	0.988
		EXP	19.1	12.8	2.1		
FORSTER 1	0.571	OBS	33	31	21	5.612	0.004 *
		EXP	27.7	41.6	15.6		
FORSTER 2	0.764	OBS	42	29	3	0.585	0.563
		EXP	43.2	26.7	4.1		
FORSTER 3	0.672	OBS	12	19	1	4.377	0.013 *
		EXP	14.5	14.1	3.4		
PORT STEPHENS	0.561	OBS	35	31	24	8.452	0.000 *
		EXP	28.3	44.3	17.3		
NEWCASTLE	0.608	OBS	24	25	11	0.946	0.609
		EXP	22.2	28.6	9.2		
SYDNEY	0.635	OBS	35	29	14	2.835	0.057
		EXP	31.5	36.2	10.4		
JERVIS BAY	0.638	OBS	42	41	15	0.756	0.526
		EXP	40	45	13		
EDEN	0.672	OBS	48	29	16	7.774	0.000 *
		EXP	42	41	10		
LAKES ENTRANCE	0.631	OBS	42	41	16	1.061	0.347
		EXP	39	46	14		
SAN REMO	0.645	OBS	41	38	14	1.073	0.343
		EXP	38.7	42.6	11.7		
POINT LONSDALE	0.707	OBS	19	20	2	1.374	0.252
		EXP	20.5	17	3.5		
CAPE PATTON 1	0.652	OBS	45	26	18	11.16	0.000 *
		EXP	37.8	40.4	10.8		
CAPE PATTON 2	0.654	OBS	40	39	12	0.275	0.763
		EXP	38.9	41.2	10.9		
APOLLO BAY	0.628	OBS	30	38	10	0.163	0.850
		EXP	30.8	36.4	10.8		
PORT FAIRY	0.639	OBS	40	44	13	0.025	0.976
		EXP	39.6	44.8	12.6		
HOBART	0.709	OBS	46	37	8	0.022	0.979
		EXP	45.7	37.6	7.7		
ANXIOUS BAY	0.602	OBS	25	21	13	3.931	0.019 *
		EXP	21.4	28.3	9.3		

Table 10. Mpi gene frequencies (p), observed (obs) and expected (exp) genotype frequencies, G-statistic and probabilities for goodness of fit to the Hardy-Weinberg distribution for samples of *S. bassensis flindersi*. * indicates significant deviation from the H-W distribution.

Figure 16: Diagram to show the location of 'tentative' subpopulations of *S. bassensis flindersi*. See text for explanation.

The Hobart sample is related genetically to those from Lakes Entrance and San Remo, but this almost certainly reflects a one way flow of larvae from the mainland across Bass Strait to Tasmanian waters.

Sillago bassensis bassensis.

In *S. bassensis bassensis* there were only ten polymorphic loci: Aat-2, Adh-1, Cat, Dia-1, Est, Gpi-1, Gpi-2, Gpi-3, Pep-C and Pgm-1. We were, however only able to use five of these in our between population comparisons. Of these five, Aat-2, Gpi-1, Gpi-2, Gpi-3 and Pep-C, only Pep-C had a frequency of less than 0.9 for its most common allele. Considerable difficulties were encountered in tissue preparation (see Methods) and in obtaining samples of this sub-species. Also, the samples from Mandurah were in poor condition and this created further problems.

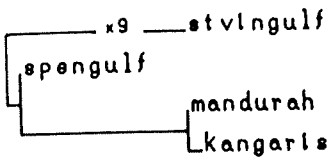
The data obtained from electrophoretic analysis (Table 7.3, Appendix 7) were used to construct dendrograms using the programs CONTML and FITCH of Felsenstein (1981, 1982). The dendrograms with the greatest likelihood are found in Figure 17.

In both cases the St. Vincent's Gulf population shows the lowest relationship to the others, with the Mandurah and Kangaroo Is. populations being the most similar. The G-tests which were carried out between all possible pairs of populations, however, showed that the genetic differences between all of these pairs of populations were significant (Table 11). Most of the difference between these populations was due to Pep-C and the allele frequencies at this locus are shown in Figure 18.

Discriminant function analysis of meristic and morphometric characters also showed large differences between the only two populations of *S. bassensis bassensis* compared, namely Mandurah and St Vincent's Gulf (Figure 7).

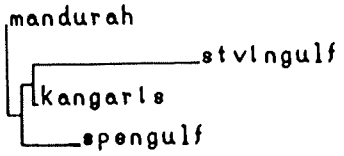
These data, taken together, suggest that the four samples of *S. bassensis bassensis* examined may be from separate sub-populations. However further work should be done before management is arranged along these lines. Further suitable polymorphic loci should be sought and samples from more localities examined.

a



SS = 0.02258 %SD = 4.75194

b



50.08058

Figure 17: Dendrograms to show the relationships between populations of *S. bassensis bassensis*. (a) CONTML plot, (b) FITCH plot.

Key: stvingulf - St Vincents Gulf
spengulf - Spencer Gulf
mandurah - Mandurah
kangaris - Kangaroo Island

otu 1	vs otu 2	g stat	d of f	prob.
stvingulf	spengulf	121.715	13	0.000 *
	kangaris	45.7856	13	0.000 *
	mandurah	81.3976	13	0.000 *
spengulf	kangaris	57.2238	10	0.000 *
	mandurah	69.2752	12	0.000 *
kangaris	mandurah	7.26545	12	.8396

Table 11. Comparisons between samples of *S. bassensis bassensis*, from four localites, by means of G-statistic (Sokal and Rohlf, 1981). Key to populations as in Figure 17. * indicates a significant result.

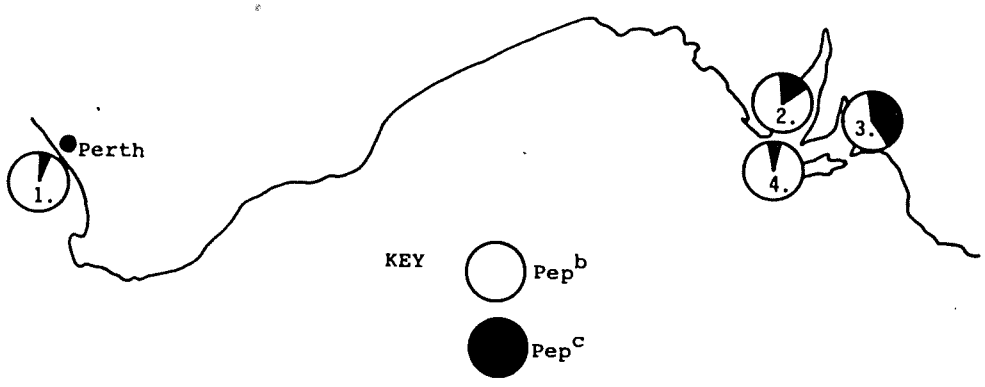


Figure 18: Frequencies of Pep^b and Pep^c in *S. bassensis bassensis* from four localities.

Key: 1. Mandurah, 2. Spencer Gulf,
3. St Vincents Gulf, 4. Kangaroo Island.

Sillago robusta

Comparisons between N.S.W. samples.

In the case of *S. robusta*, 42 enzyme systems were investigated in the pilot study. These encode about 65 genetic loci. The data are found in Table 3.6, Appendix 3. The pilot study used only specimens from N.S.W. The level of polymorphism was low: only 12 out of the 65 loci examined showed any polymorphism. These suspected polymorphic loci were: Gda-2, G6pdh-1, Gpi-1, Gpi-2, Gpi-3, Idh-1, Idh-2, Mpi, Me-1, Pgm-1, Pgm-2 and Sdh.

Seven polymorphic loci were selected for the ease of genetic interpretation; there were: Gpi-1, Gpi-2, Gpi-3, Mpi, Idh-1, Pgm-1 and Sdh (See Appendix 6 for a description of the enzyme banding pattern of these polymorphic loci). Of these only in the latter three cases was the frequency of the most common allele less than 0.9.

The polymorphic loci were used to compare seven samples which were collected from six localities. The detailed data obtained are found in Table 7.4, Appendix 7.

We used Felsenstein's PHYLIP package to compare the different samples. The results of the comparisons, which were made using CONTML and FITCH, are shown in Figure 19. Note that in both cases the two kinds of plot are very similar but in neither case are the samples assorted according to geographic proximity.

Table 12 gives the results of pairwise tests using the G-statistic. The sample from Sandon Bluffs is different from all the others. There are no known barriers to gene flow in this area so it seems likely that we are seeing the results of patchy recruitment (see discussion relating to *S. bassensis flindersi*). Further work needs to be done to investigate this fully.

Comparisons between samples from Sydney (N.S.W.), Rottnest Island (W.A.) and Tasman Point, The Gulf of Carpentaria (N.T.).

In these comparisons only 15 enzyme systems were investigated. These encode 27 gene loci. Large differences were found between the samples. Table 13 gives the differences that were found to be fixed in species pair comparisons.

In comparisons between N.T. and W.A. samples there were

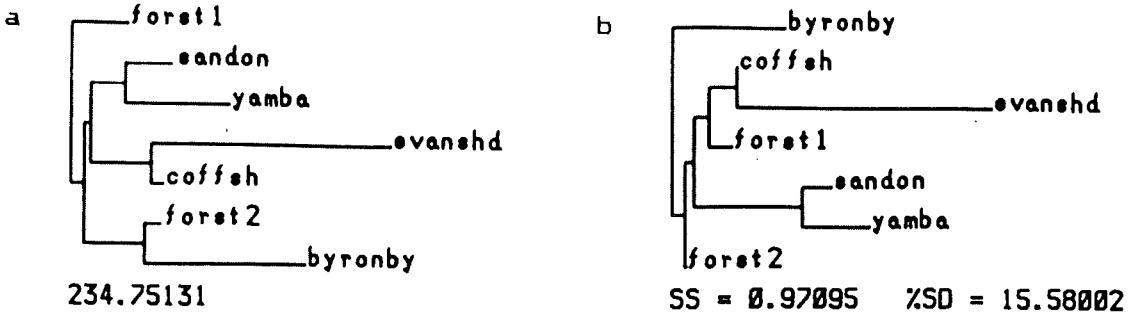


Figure 19: Dendrograms to show the relationships between *S. robusta* samples from northern N.S.W. (a) CONTML plot, (b) FITCH plot.

Key: forst1 - Forster (1/10/85)
forst2 - Forster (20/5/86)
sandon - Sandon Bluff
coffsh - Coffs Harbour
yamba - Yamba
evanshd - Evans Head
byronby - Byron Bay

otu 1	vs otu 2	g stat	d of f	prob.
byronby	evanshd	27.0711	21	.1685
	yamba	25.4177	21	.2295
	sandon	92.6687	20	0.000 *
	forst1	23.0418	20	.2867
	forst2	20.8548	20	.4057
	coffsh	21.2262	20	.3839
evanshd	yamba	30.5317	20	.0617
	sandon	87.3607	20	0.000 *
	forst1	28.2955	19	.0779
	forst2	35.2964	20	.0186 *
	coffsh	20.2637	20	.4415
yamba	sandon	56.9542	18	0.000 *
	forst1	11.1661	16	.7991
	forst2	27.624	19	.0909
	coffsh	14.4734	19	.7554
sandon	forst1	67.8053	16	0.000 *
	forst2	97.2999	17	0.000 *
	coffsh	73.7703	17	0.000 *
forst1	forst2	16.034	17	.5214
	coffsh	9.98396	17	.9043
forst2	coffsh	22.4646	18	.2120

Table 17. Comparisons between samples of *S. robusta*, from six localities, by means of the G-statistic (Sokal and Rohlf, 1981). Key to samples as in Figure 19. * indicates a significant result.

Enzyme	Tissue	Number of Loci	Species Pair Differences		
			NT/WA	NT/NSW	WA/NSW
AAT	L	4	Aat-1	Aat-1	-
			-	Aat-2	Aat-2
			-	Aat-3	Aat-3
			Aat-4	Aat-4	Aat-4
ADA	L	1	Ada	-	Ada
ADH	L	1	Adh	Adh	Adh
EST	L	1	-	Est	Est
GDA	L	1	-	Gda	Gda
GPI	L	1	-	-	-
	M	3	-	-	Gpi-2
			-	Gpi-3	Gpi-3
IDH	L	1	Idh-2	Idh-2	-
	M	1	-	-	-
LDH	L or M	1	-	-	-
MDH	M	3	-	Mdh-2	Mdh-2
			-	Mdh-3	Mdh-3
ME	M	2	Me-2	Me-2	
MPI	M	1	-	-	-
PEP-C	L	1	-	PepC	PepC
PEP-D	L	2	-	PepD-1	PepD-1
PGM	L or M	2	-	-	-
SOD	M	1	Sod	Sod	-
TOTAL NUMBER		27	7	16	13

TABLE 13: The enzymes examined, the tissues used, the numbers of loci investigated and the species pair differences (diagnostic loci) found in comparisons between *S. robusta* from N.S.W., W.A. and N.T.

Plate 4. *Sillago robusta* (N.S.W.)

Plate 5. *Sillago robusta* (W.A.)

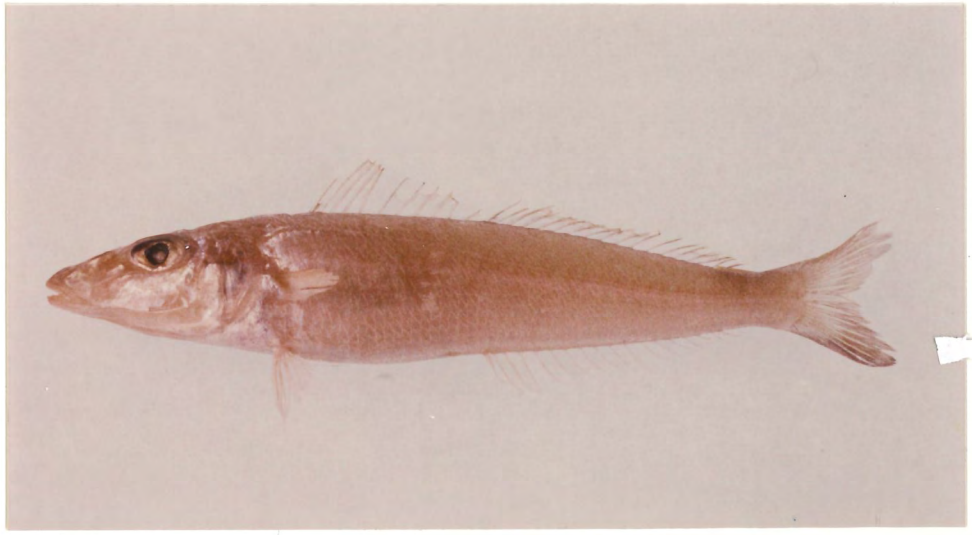


Plate 6. *Sillago robusta* (Gulf, N.T.)

7/27 fixed differences; the N.T./N.S.W. comparison revealed 16/27 fixed differences; and the W.A./N.S.W. comparison showed 13/27 such differences. These differences are much greater than those we found in comparisons between *S. bassensis bassensis*, *S. bassensis flindersi* and *S. vittata*. Differences between the samples were also apparent in the soluble muscle proteins when visualised after isoelectric focusing (Figure 20).

In his review of the sillaginids, McKay (1985) found geographic variation in *S. robusta*. He said that this species is divided into two distinct populations, one on the east coast and the other on the northern and western coasts of the continent. The main differences between the two groups were:

(a) the shape of the swimbladder,
(b) the development of the first dorsal spine keel, which was more pronounced in the eastern population;
and (c) the relationship between the posterior extension of the swimbladder and the posterior third of the modified caudal vertebrae.

It is apparent that McKay believed that these two forms were probably sub-species; he was awaiting additional specimens from northern Australia and the results of a full osteological comparison before providing a sub-specific name. We have not made a morphological study of the specimens we examined except to the extent necessary to distinguish them from other whiting species. However, we did notice some differences in the colour and shape of the fish from the different localities. The fish from eastern Australia were of darker colour than those from the other areas, and those from the N.T. were particularly pale. There were also some differences in body shape (see Plates 4-6).

The differences that we found between these fish suggest that there are three groups of fish, not two. The large size of the genetic differences indicates that these groups may belong to separate species. But how different do allopatric populations need to be before they can be considered separate species? Richardson *et al.* (1986) say that allopatric populations of vertebrates can be considered to be separate species, with a high degree of confidence, if there are fixed differences at more than 20% of the loci examined. In each of the population pairs that we considered the proportion of alleles that show fixed differences exceeds this level; in the case of the N.S.W./N.T. comparison, fixed differences occur at almost 60% of the loci examined. If this criterion is used there is no doubt that each population belongs to a distinct species. Figure 21 shows the distributions

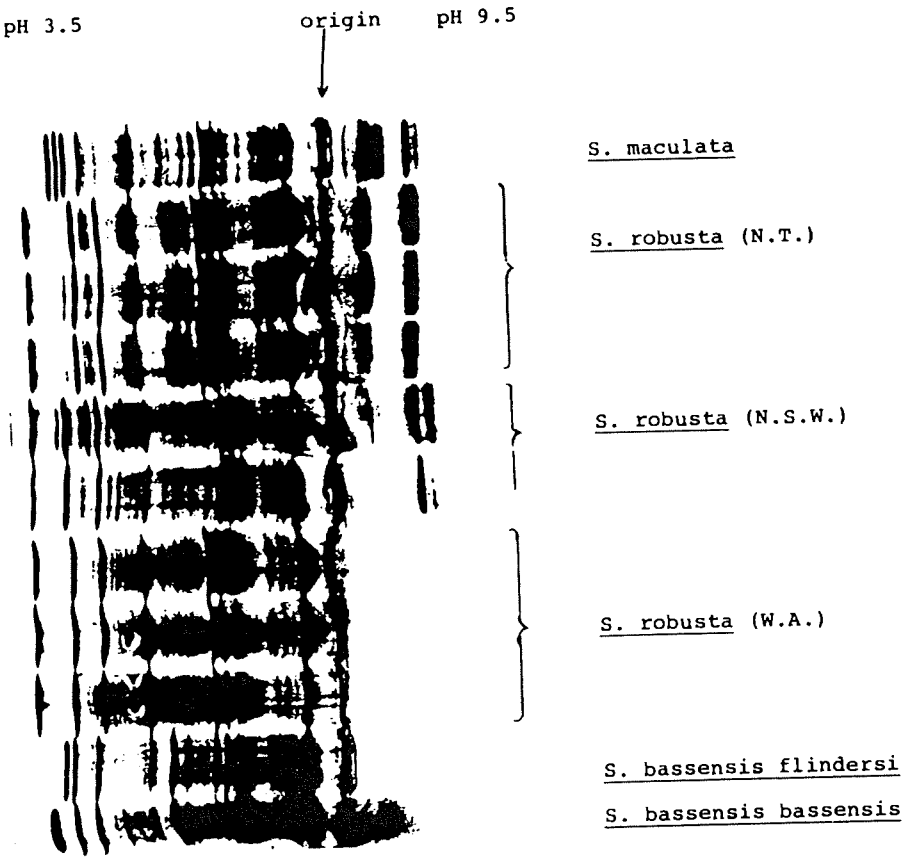


Figure 20: Isoelectric focusing gel of soluble muscle proteins from *S. maculata* (N.S.W.), *S. robusta* (N.T., N.S.W., W.A.), *S. bassensis bassensis* and *S bassensis flindersi*.

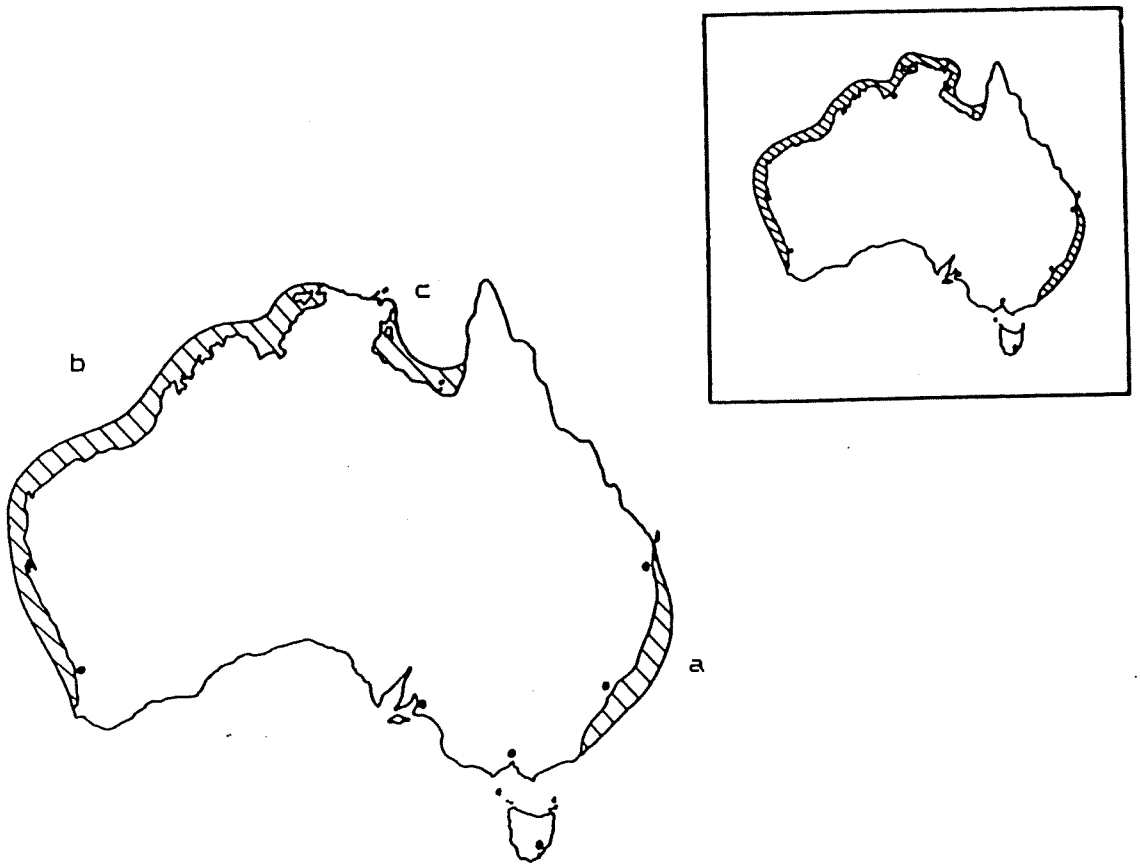


Figure 21: Maps to show the distribution of *S. robusta* (a) Eastern form, (b) Western form, (c) gulf form. The inset shows the distribution described by McKay (1985).

of the three "forms" of *S. robusta* as proposed by us; McKay's (1985) distribution is shown in the inset. Further work should be done on these "forms" to delimit their distributions.

Sillago maculata

Pilot Study

Thirty-nine enzyme systems were examined in *S. maculata maculata*. These encode about 60 loci. Of these loci 13 showed polymorphism (see Table 3.7, Appendix 3). The polymorphic loci were: Aat-3, Cat-L, Enol, Gpi-1, Gpi-2, Gpi-3, Idh-1, Idh-2, Mdh-1, Mpi, Pgd, Pgm-1, and Sdh. These were not fully investigated but the data will provide a good basis for further studies on this sub-species, particularly ones in which populations are to be compared.

Comparisons between *S. maculata maculata*, and *S. maculata burrus*.

Comparisons were made between *S. maculata maculata* from N.S.W. and *S. maculata burrus* from Mandurah (W.A.) and the Gulf of Carpentaria (N.T.). Eighteen enzyme systems which encode 23 gene loci were examined. These were: AAT, ADA, ADH, EST, GDA, GPI, IDH, LDH, MDH, ME, MPI, PEPC, PEPD, PGD, PGM, SDH, SOD and XDH. The loci Aat-2, Adh-1, Gpi-3, Gpi(L), Gpt, Idh-2, Me-1, Mpi, Pgd, Pgm-1 and Pgm-2 were polymorphic in *S. maculata burrus* (see Table 3.8, Appendix 3). No fixed differences were found.

When the soluble muscle proteins of these sub-species were compared after isoelectric focusing, some differences were apparent (Figure 22). These differences are at least as great as those recorded for *S. robusta* but they have not been fully investigated.

The lack of genetic differences between these sub-species was unexpected, especially in the light of our findings with *S. bassensis* and *S. robusta*.

Sillago maculata maculata and *S. maculata burrus* are two of the three sub-species of *S. maculata*. The other, *S. maculata aeolus*, is not known from Australian waters. McKay distinguishes the sub-species on the basis of swim bladder morphology. We have made no morphometric comparisons between the sub-species but we had no difficulty in distinguishing between them on the basis of the pattern of the dark blotches on their bodies and their body shape (Plates 7 and 8).

We are unable, on the basis of our fairly limited genetic data, to say whether *S. maculata maculata* and

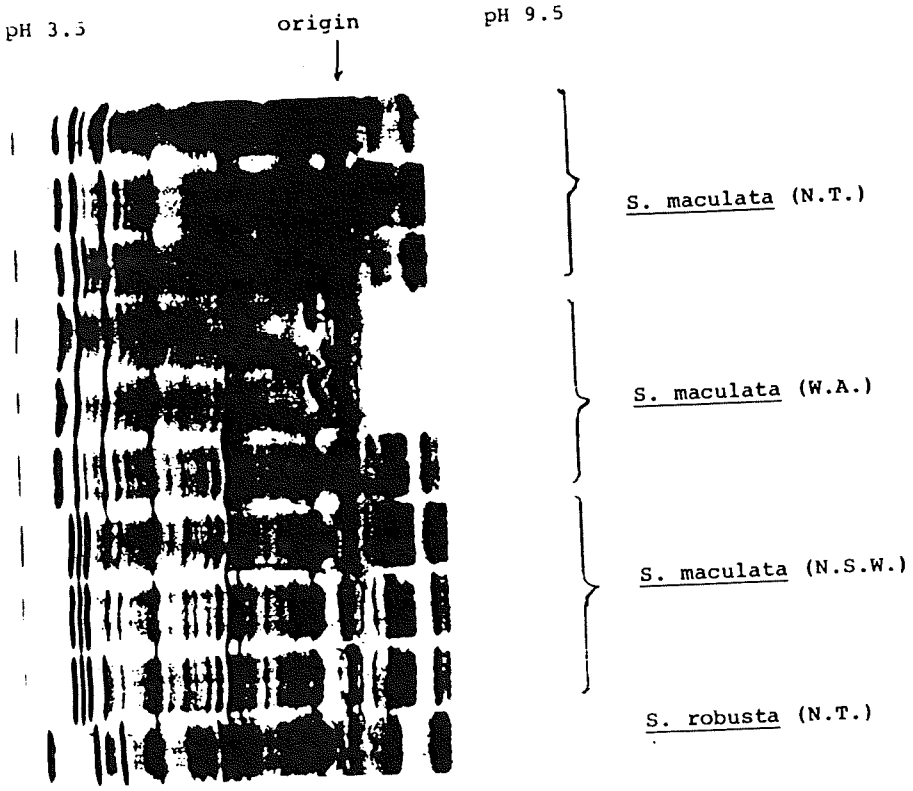


Figure 22: Isoelectric focusing gel of soluble muscle proteins from *S. maculata* (N.T., W.A., N.S.W.) and *S. robusta* (N.T.).

Plate 7. *Sillago maculata maculata*

Plate 8. *Sillago maculata burrus*

S. maculata burrus are 'good' (i.e. useful) sub-species. A great deal more work, e.g. further sampling and increasing the number of loci examined, is necessary before we would be prepared to make any recommendation on this matter.

Sillaginodes punctata

Considerable difficulties were encountered in obtaining good samples of this species throughout its range. We were able to obtain limited samples from southern Victoria and South Australia. No samples were obtained from Western Australia (see Table 2.1, Appendix 2).

Thirty-eight enzyme systems were investigated in the pilot study, encoding for 46 presumed genetic loci. Of these loci, 10 showed possible polymorphism. These loci were: Damox, Dia-2, Gpi-1, Gpi-2, Gpi-3, Gpt, Me, Pgd, Pgm-1 and Pgm-2.

The polymorphic loci used in the limited investigation into the population structure of *S. punctata* were: Gpi-1, Gpi-2, Gpi-3, Pgm-1 and Pgm-2 from muscle, and Pgd and Gpt from liver. Of these, only Pgd and Gpt had frequencies of less than 0.9 for the most common allele.

However, due to the poor condition of the samples from Victoria* and some of the samples from South Australia, only three sampling sites were screened successfully for Pgd (Adelaide, Swan Bay and Corner Inlet). For Gpt, six of the seven sampling sites were successfully screened.

Due to these missing data, several gene frequency sets (Tables 7.5, 7.6 and 7.7, Appendix 7) were input into Felsenstein's (1981, 1982) CONTML and FITCH programs to construct dendrograms. The dendrograms with the greatest likelihoods are shown in Figure 23. In this figure (a) and (b) are based on the loci Gpt, Gpi-1, Gpi-2, Gpi-3, Pgm-1 and Pgm-2; (c) and (d) on Gpt, Gpi-1, Gpi-2, Gpi-3, and Pgm-1, and (e) and (f) on Gpt, Gpi-1, Gpi-2, and Gpi-3.

* These samples were of juveniles less than 1 year old. Due to their small size, handling difficulties were encountered. The samples thawed completely arrival to the laboratory, and thawed again before dissection. This resulted in the loss of Pgd activity in the liver tissue.

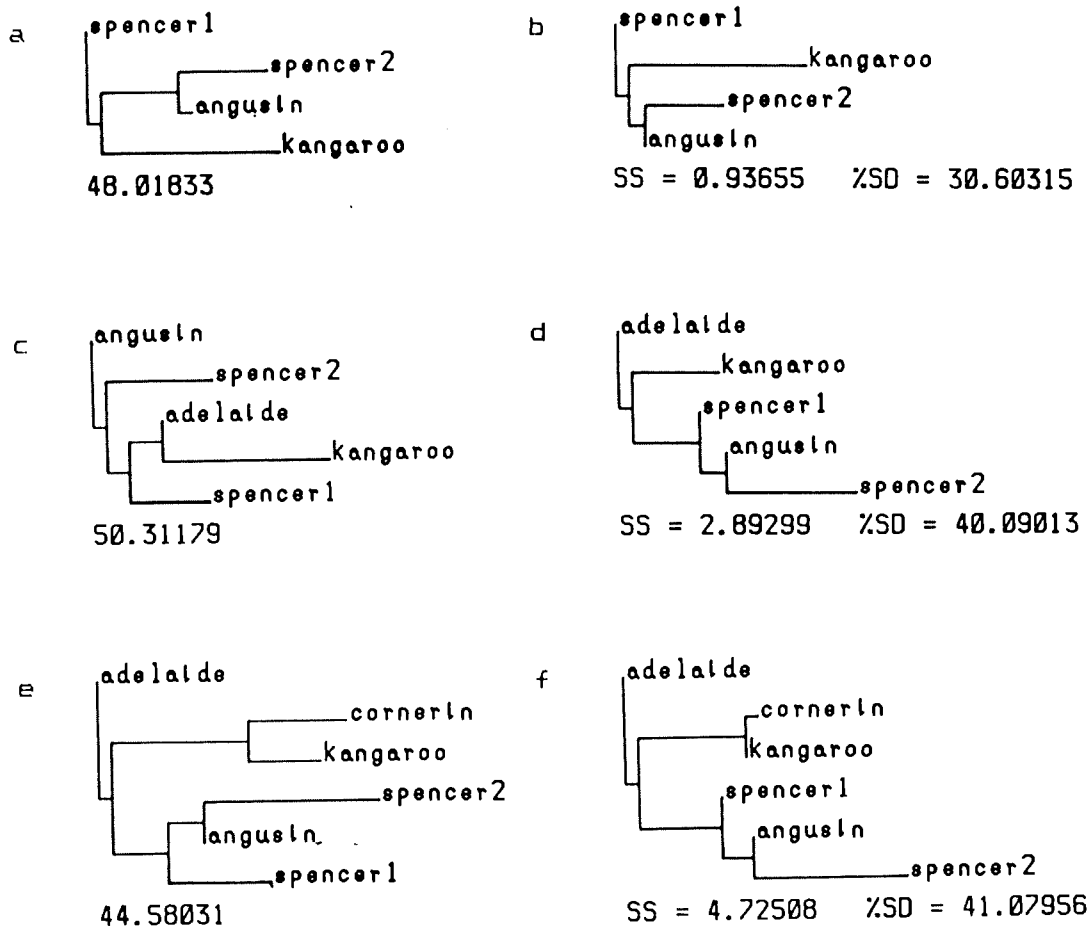


Figure 23: Dendrograms to show the relationships between *Sillaginodes punctata* samples from different localities. The plots are based on three different data sets (see text for details). Plots a, c and e were prepared using CONTML; b, d and f using FITCH (Felsenstein, 1981, 1982).

Key: spencer1 - Upper Spencer Gulf (1/11/85)
spencer2 - Upper Spencer Gulf (3/11/85)
adelaide - Port Adelaide
angusin - Angus Inlet
kangaroo - Kangaroo Island
cornerin - Corner Inlet

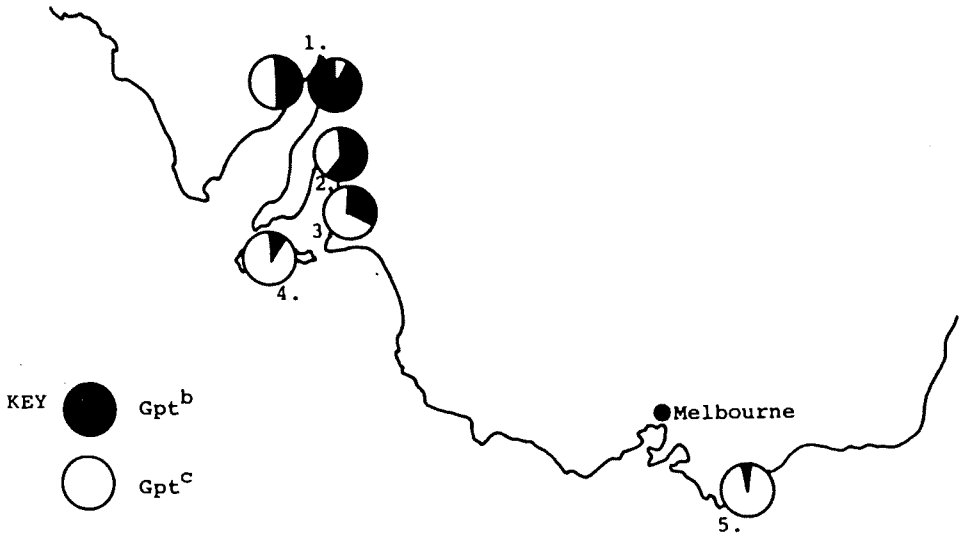


Figure 24: Frequencies of Gpt ^{b} and Gpt^{c} in *S. punctata* from five localities.

Key: 1. Upper Spencer Gulf; 2. Angus Inlet; 3. Port Adelaide; 4. Kangaroo Island; 5. Corner Inlet.

a

otu 1	vs otu 2	g stat	d of f	prob.
spencer1	spencer2	42.6816	16	.0003 *
	angusin	56.6322	16	0.000 *
	kangaroo	40.1143	16	.0007 *
spencer2	angusin	25.3548	10	.0047 *
	kangaroo	69.293	13	0.000 *
angusin	kangaroo	57.4495	12	0.000 *

b

otu 1	vs otu 2	g stat	d of f	prob.
adelaide	spencer1	15.7695	12	.2020
	spencer2	29.394	6	.0001 *
	angusin	13.404	5	.0199 *
	kangaroo	12.6049	8	.1262
spencer1	spencer2	41.246	12	0.000 *
	angusin	41.481	12	0.000 *
	kangaroo	34.8603	12	.0005 *
spencer2	angusin	14.4391	6	.0251 *
	kangaroo	64.0603	9	0.000 *
angusin	kangaroo	39.4584	8	0.000 *

c

otu 1	vs otu 2	g stat	d of f	prob.
adelaide	spencer1	8.80659	9	.4553
	spencer2	29.174	5	0.000 *
	angusin	11.2049	4	.0244 *
	kangaroo	10.2038	5	.0697
	cornerin	23.6518	6	.0006 *
spencer1	spencer2	29.4334	9	.0005 *
	angusin	18.5132	9	.0297 *
	kangaroo	27.2406	9	.0013 *
	cornerin	81.4942	9	0.000 *
spencer2	angusin	13.2534	5	.0211 *
	kangaroo	60.5387	6	0.000 *
	cornerin	119.342	6	0.000 *
angusin	kangaroo	32.588	5	0.000 *
	cornerin	75.8278	6	0.000 *
kangaroo	cornerin	19.2785	7	.0074 *

Table 14. Comparisons between samples of *S. punctata* by means of the G-statistic (Sokal and Rohlf, 1981). Key to samples as in Figure 23.
(a) Comparisons based on Gpt; Gpi 1-3, Pgm-1 and Pgm-2.
(b) Comparisons based on Gpt; Gpi 1-3 and Pgm-1.
(c) Comparisons based on Gpt and Gpi 1-3.

There is an increasing number of samples included in the analyses as the number of loci considered is decreased. However all analyses include Gpt^c which was the only locus at which the frequency of the most common allele was less than 0.900.

Even though there are different numbers of samples included in each analysis the results of all of them are very similar. The samples from Kangaroo Island and Corner Inlet are genetically more similar to each other then they are to the samples from the two gulfs.

A similar trend is seen when the frequencies of alleles at the Gpt locus are considered. In this case the frequency of Gpt^c is very low in both the Kangaroo Island and Corner Inlet samples and high in the samples from the gulfs (Figure 24).

It is unfortunate that insufficient data was obtained from the Swan Bay (Vic.) samples to include it in any of these analyses.

Care must be taken, however, in the interpretation of similarities based on one locus especially in a case like this. The gulfs, especially in their upper reaches, are well known for their high salinities and temperatures. It is likely that the environments of fish from the gulfs would be quite different from those that live in more open embayments which are not so subject to evaporative water loss. The differences observed, therefore, could be due to chance, selection or genetic relatedness. We have no data to enable us to decide between these three options.

The data were compared by means of G-tests which use all the available data. The same three data sets as described above were used. Significant differences were found in all comparisons except two. These two were Adelaide with Spencer Gulf 1 and Adelaide with Kangaroo Island. These non-significant results were consistent in comparisons using the two larger data sets. The results of these G-tests are found in Table 14.

Thus there are indications that there may be sub-structuring within the population of *S. punctata* but further investigations must be completed before any definite conclusions are reached. These investigations should include comprehensive sampling of the species throughout its range, refinement of collecting and laboratory procedures to reduce damage to the enzymes, and a search for further enzyme polymorphism.

PILOT STUDIES ON OTHER SPECIES

The following brief results and comments are for species that were not investigated in detail in this programme. They are included because the data will provide a useful starting point for further such studies on these species.

Sillago vittata

The pilot study on *S. vittata* included 27 enzymes which were studied in liver and muscle tissue. Four of these showed no activity; the remaining 23 encode 44 presumed gene loci. These data are found in Table 3.5, Appendix 3. Only 9 of these loci; Ald, Cat, Gda, Gpi-2, Gpi-3, Idh-2, Mpi, Pgd, and Sdh were polymorphic. Except in the cases of Gpi-2, Gpi-3 and Mpi, the resolution of these polymorphic differences was poor. Unless further polymorphic loci can be found and/or resolution of those already detected is improved, there is little potential for the use of such differences in the study of the population structure of these species.

Sillago ciliata

Forty-nine enzymes were investigated in the liver, muscle, heart and eye lens tissue of *S. ciliata*. Of these, two showed no activity in any of the tissues studied but the remaining 47 encode about 81 gene loci. Twenty three loci displayed polymorphism (for details see Table 3.9, Appendix 3). Although most of these loci require further investigation to determine their usefulness in future population studies, Enol-1, Idh-1, Mpi, Pgd and Pgm-1 are likely to be useful because in each case the frequency of the most common allele was less than 0.9 in the samples we used in our pilot study.

Sillago schomburgkii

Only 15 enzymes were investigated in the liver and muscle tissue of *S. schomburgkii*. Four of these, Ak-2, Est-2, Mpi, and Pgd were polymorphic. The details of these data are found in Table 3.11, Appendix 3. Further work must be done if the true potential for the use of isozyme polymorphisms in population studies on this species is to be established.

Sillago analis

Only a very small amount of work was done on a few specimens of *S. analis*. Eleven enzymes were studied in

liver and muscle tissue. Four of them showed no activity, the remaining éight encode about 12 gene loci. Four of the loci, Gpi-2, Gpi-3, Pep (FP) and Pgm showed polymorphism, but activity was poor in the case of Pep (FP). The details of these data are found in Table 3.10, Appendix 3. Further work must be done to determine whether isozyme polymorphisms are likely to be useful in the investigation of the population structure of *S. analis*.

McKay (1985) regards *S. ciliata* and *S. analis* as sibling species. In this preliminary investigation we found that there were fixed genetic differences between these two species at four of the eleven loci studied; Gpi-1, Gpi-2, Ldh(L) and Pgm(M). Although the numbers of individuals examined so far are small, it is likely that, despite their morphological similarities, the genetic differences between them are considerable.

LITERATURE CITED

Albrecht, G.H. (1978). Some comments on the use of ratios. Syst. Zool. 27: 67-71.

Allendorf and Utter (1979). Fish Physiology Vol VIII, Chapter 8, 407-454 Academic.

Anon. (1981). Popular marine species. In: Recreational Fishing Guide, 3-6. South Australian Department of Fisheries, Adelaide.

Anon. (1984). Enzyme nomenclature 1984. Recommendations of the nomenclature committee of the international union of biochemistry on the nomenclature and classification of enzyme-catalysed reactions. Academic.

Atchley, W.R., Gaskins, C.T. & Anderson, D. (1976). Statistical properties of ratios. I. Empirical results. Syst. Zool. 25: 137-148.

Avise,J.C. abd Kitto,G.B. (1973). Phosphoglucose Isomerase Gene Duplication in the Bony Fishes: An Evolutionary History. Biochemical Genetics 8 (2): 113-132.

Bowerman,M. (1984). "By-catch" pays the bills for Clarence prawners. Australian Fisheries 43(7) : 14-16.

Cresswell, G.R., Ellyett, C., Legeckis, R. and Pearce, A.F. (1983). Nearshore features of the East Australian Current. Aust. J. Mar. Freshw. Res. 34: 105-114.

Denman, K.L. and Powell, T.M. (1984) Effect of physical processes on planktonic ecosystems in the coastal ocean. Oceanog. Mar. Biol. Ann. Rev. 22: 125-168.

Dillon, W.R. & Goldstein, M. (1984). Multivariate analysis - Methods and Applications. Publ. J. Wiley & Sons, Inc. USA. ISBN 0-471-08317-8. p412.

Dodson, P. (1978). On the use of ratios in growth studies. Syst. Zool. 27: 62-67.

Fasham, M.J.R. (1978). The statistical and mathemetical analysis of plankton patchiness. Oceanogr. Mar. Biol. Ann. Rev. 16: 43-79.

Felsenstein, J. (1981). Evolutionary trees from gene frequencies and quantative characters: finding maximum likelihood estimates. Evolution 35: 1229-1242.

Felsenstein, J. (1982). Numerical methods for inferring evolutionary trees. Quarterly Review of Biology 57: 379-404.

Godfrey, J.S., Cresswell, G.R. Golding. T.J., Pearce, A.F. and Boyd, R. (1980). The separation of the East Australian Current. J. Phys. Oceanog. 10: 430-440.

Gorman, T.B. *et al* (1985). Kapala Cruise Report No. 94. Part II. (Division of Fisheries, Dept. of Agriculture NSW).

Gorman, T.B. *et al* (1986). Kapala Cruise Report No. 97. Part II. (Division of Fisheries, Dept of Agriculture NSW).

Griffiths, R.A. (1986). A low cost data-collecting system. Australian Fisheries 45(3): 34-5.

Harris,H and Hopkinson,D.A. (1976-1978). Handbook of enzyme electrophesis in human genetics. North Holland, Amsterdam.

Haury, L.R., Wiebe, P.H., Orr, M.H. and Briscoe, M.G. (1983). Tidally generated wave packets and their effects on plankton in Massachusetts Bay. J. Mar. Res. 41: 65-112.

Hillis, D.M. (1984). Misuse and modification of Nei's genetic distance. Syst. Zool. 37 (2): 238-240.

Hills, M. (1978). On ratios - a response to Atchley, Gaskins and Anderson. Syst. Zool. 27: 61-62.

Hobday, D.K. and Wankowski, W.J. (1986). Age determination and growth of school whiting. Internal Report 130. Marine Science Laboratories, Queenscliffe, Victoria.

Jones, K. (1980). Research on the biology of spotted (King George) whiting in South Australian waters. *SAFIC* 4(i): 3-7.

Mackas, D.L., Denman, K.L. and Abbott, M.R. (1985). Plankton patchiness: biology in the physical vernacular. Bulletin of Marine Science 37:652-674.

McKay, R.J. (1985). A revision of the fishes of the Family Sillaginidae. Mem. Qld Mus. 22(1): 1-73.

Nie, N.H., Hull, C.H., Jenkins, J.G., Steinbrenner, K. & Bent, D.H. (1975). Statistical package for the Social Sciences. McGraw-Hill, NY.

Reist, J.D. (1985). An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Can. J. Zool. 63: 1429-1439.

Richardson, B.J., Baverstock, P.R. and Adams, M. (1986). Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, Australia.

Rochford, D.J., (1975). Oceanography and its role in the management of aquatic ecosystems. Proc. Ecol. Soc. Aust. 8 : 67-83.

Shaklee, J.B. and Keenan, C.P. (1986). A practical laboratory guide to the techniques and methodology of electrophoresis and its application to fish fillet identification. Report 177 (CSIRO Marine Laboratories).

Shaw, C.R. and Prasad, R. (1970). Starch gel electrophesis - a compilation of recipes. Biochem. Genet. 4 : 297-320.

Smith, D.C. (1985). Assessment of the New South Wales Red Spot Whiting Fishery. Fisheries Research Institute Dept. of Agriculture NSW Internal Report No.3.

Sokal, R.R. and Rohlf, F.J. (1973). Biostatistics. (Freeman San Francisco).

Thorpe, R.S. (1975). Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the Ringed Snake *Natrix natrix* (L.) Bio. J. Linn. Soc. 7: 27-43.

Winstanley, R.H. (1983). Fishery Stiuation Report: School Whiting. Commercial Fisheries Report, Victoria Number 9.

APPENDIX 1 : WHITING SPECIES OCCURRING AROUND AUSTRALIA.

TABLE 1.1 : Whiting Species Occurring Around Australia.

Scientific Name	Common Name	Distribution
Sillago analis	Golden-Lined Whiting	QLD, NT, WA.
Sillago bassensis bassensis	Western School or Transparent Whiting	WA, SA, W.VIC.
Sillago bassensis flindersi	Eastern School or Red Spot Whiting	Sth QLD, NSW, VIC, TAS, SA.
Sillago ciliata	Sand Whiting	QLD, NSW, VIC,TAS
Sillago lutea.	Mud Whiting	NT, WA.
Sillago maculata burrus	Western Trumpeter Whiting	NT, WA.
Sillago maculata maculata	Trumpeter Whiting	QLD, NSW, VIC.
Sillago robusta (Eastern)	Stout Whiting	Sth QLD, NSW.
Sillago robusta (Western)		NT, WA.
Sillago schomburgkii	Yellow Fin Whiting	WA, SA.
Sillago sihama.	Northern Whiting	NT, WA.
Sillago vittata	Banded Whiting	WA.
Sillaginodes punctata	King George Whiting	WA, SA, VIC.

(From McKay,1985)

[* As yet we have been unable to obtain specimens of these species.]

Around Australia, there are 10 species and 3 sub-species of whiting.

By state these are:

NSW (4)
- *Sillago bassensis flindersi*
- *Sillago ciliata*
- *Sillago maculata maculata*
- *Sillago robusta* (Eastern Form)

VIC (5)
- *Sillago bassensis bassensis*
- *Sillago bassensis flindersi*
- *Sillago ciliata*
- *Sillago maculata maculata*
- *Sillaginodes punctata*

SA (4) and now
- *Sillago bassensis bassensis*
- *Sillago bassensis flindersi*
- *Sillago schomburgkii*
- *Sillaginodes punctata*

WA (9)
- *Sillago analis*
- *Sillago bassensis bassensis*
- *Sillago lutea*
- *Sillago maculata burrus*
- *Sillago robusta* (Western Form)
- *Sillago schomburgkii*
- *Sillago sihama*
- *Sillago vittata*
- *Sillaginodes punctata*

NT (5)
- *Sillago analis*
- *Sillago lutea*
- *Sillago maculata burrus*
- *Sillago robusta* (Western Form)
- *Sillago sihama*

QLD (5)
- *Sillago analis*
- *Sillago bassensis flindersi*
- *Sillago ciliata*
- *Sillago maculata maculata*
- *Sillago robusta* (Eastern Form)

TAS (2)
- *Sillago bassensis flindersi*
- *Sillago ciliata*

APPENDIX 2 : COLLECTION DATA FOR WHITING SPECIES.

Key :
F = female, M = male, J = juvenile
SL = standard length
- = no data

TABLE 2.1 : Sample Collection Data For King George Whiting, *Sillaginodes punctata.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
S.A.				
Fowlers Bay	28/ 2/84	13	-	-
Port Adelaide	13/ 9/84	20	20J	11.4 to 17.0
Upper Spencer	1/11/85	49	21F:11M:17J	17.7 to 25.4
Gulf	3/11/85	37	7F:21M: 9J	14.2 to 26.0
Kangaroo Is	20/11/85	26	26J	11.4 to 16.1
Angus Inlet	13/ 2/86	60	60J	5.6 to 9.1
VIC				
Queenscliff	9/ 4/85	100	100J	7.7 to 20.5
Western Pt Bay	10/ 4/85	20	20J	-
Shallow Inlet	12/ 4/85	100	100J	7.2 to 19.1
Corner Inlet	13/ 4/85	83	83J	7.0 to 20.4

TABLE 2.2 : Sample Collection Data For Transparent Whiting, *Sillago bassensis bassensis.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
S.A.				
Kangaroo Is	1/ 6/84	43	24F: 8M:11J	12.5 to 22.1
St Vin. Gulf	11/ 6/84	108	60F:25M:23J	13.1 to 20.8
Spencer Gulf	5/84	110	41F: 4M:65J	10.6 to 17.9
Anxious Bay	13/ 5/85	3	1F: 1M	15.7 to 17.9
W.A.				
Mandurah	13/ 5/85	110	-	8.8 to 17.2
Rottnest Is	27/ 8/85	21	6F: 9M: 4J	13.9 to 22.5

TABLE 2.3 : Sample Collection Data For Red Spot Whiting, *Sillago bassensis flindersi.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
N.S.W.				
Byron Bay	25/ 5/86	40	22F:12M: 6J	12.0 to 19.6
Evans Head	25/ 5/86	45	18F:24M: 3J	12.1 to 16.5
Yamba	7/ 6/84	200	-	12.5 to 21.7
"	22/ 5/86	33	25F: 7M: 1J	11.7 to 18.2
"	23/ 5/86	53	29F:20M: 4J	8.9 to 18.0
Nth Solitary Is	10/10/85	100	25F:36M:39J	10.7 to 16.8
Coffs Harbour	2/ 4/85	49	25F:24M	13.1 to 21.4
"	21/ 5/86	43	21F:22M	13.0 to 21.1
Sandon Bluff	5/ 6/85	7	5F: 1M: 1J	11.2 to 20.3
"	21/ 5/86	43	21F:22M	13.0 to 21/1
Wooli	11/10/85	96	41F:51M: 4J	11.7 to 18.5
Camden Heads	2/10/85	100	34F:44M:12J	8.9 to 20.3
Sth Camden Hds	2/10/85	43	19F:12M:12J	8.6 to 15.9
Crowdy Head	4/84	10	5F: 5M	10.5 to 16.9
Forster	1/10/85	100	15F:21M:66J	8.2 to 21.2
"	5/ 6/85	80	30F:45M: 5J	8.0 to 21.8
"	20/ 5/86	32	18F: 9M: 5J	8.4 to 20.4
Pt Stephens	4/84	10	4F: 1M	15.7 to 20.1
"	11/ 4/85	103	14F:13M:76J	7.4 to 15.7
Stockton Bt	11/ 4/85	62	20F:29M:13J	9.6 to 19.6
Broken Bay	12/ 4/85	78	31F:46M: 1J	13.3 to 21.8
Sydney	11/ 4/84	5	5J	4.2 to 5.8
"	13/ 6/84	12	-	7.9 to 10.3
" markets	27/ 9/84	10	-	17.0 to 23.0
Jervis Bay	9/ 8/84	138	47F:91M	12.6 to 20.2
Eden	22/ 6/84	197	103F:92M: 2J	12.0 to 22.3
VIC				
Lakes Entrance	18/ 6/84	118	62F:55M: 1J	11.5 to 21.5
San Remo	29/ 5/84	156	59F:92M: 5J	13.3 to 22.3
Pt Lonsdale	20/ 3/84	15	7F: 6M	16.6 to 21.3
"	21/ 3/85	42	21F:21M	14.3 to 19.5
Apollo Bay	12/ 9/85	79	40F:38M: 1J	12.6 to 18.5
Cape Patton	30/ 9/85	100	62F:38M	15.1 to 23.2
"	30/ 9/85	81	49F:32M	15.9 to 23.5
"	30/ 9/85	10	7F: 3M	17.1 to 22.6
Port Fairy	11/86	239	69F:166M:3J	10.7 to 22.0
TAS				
Hobart	17/ 5/84	215	95F:108M:12J	14.0 to 24.8
S.A.				
Kangaroo Is	12/86	< 60	-	-
Anxious Bay	18/ 3/86	60	44F:11M: 1J	10.9 to 15.4

TABLE 2.4 : Sample Collection Data For Banded Whiting, *Sillago vittata.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
W.A.				
Mandurah	13/ 5/85	8	8F	15.6 to 24.0
"	12/ 3/86	32	16F: 4M:12J	12.0 to 15.0
Rottnest Is	27/ 8/85	4	1F: 3M	20.9 to 24.5

TABLE 2.5 : Sample Collection Data For Stout Whiting, *Sillago robusta.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
QLD				
Coalun	10/ 9/84	1	1M	16.9
N.S.W.				
Byron Bay	25/ 5/86	76	16F: 6M:54J	11.2 to 15.7
Evans Head	25/ 5/86	74	3F: 3M:68J	10.4 to 14.0
Yamba	22/ 5/86	60	5F: 3M:52J	10.4 to 15.5
Coffs Harbour	26/ 3/85	88	17F:13M:58J	10.3 to 16.0
Sandon Bluff	5/ 6/85	105	4F:65M:32J	7.0 to 15.0
Wooli	1/10/85	1	1J	9.9
Forster	5/ 6/85	10	7F: 2M: 1J	7.8 to 14.5
"	1/10/85	67	67J	6.1 to 12.0
"	20/ 5/86	141	8F: 7M:124J	7.6 to 16.7
Sydney	20/ 9/83	38	-	9.3 to 14.9
"	11/ 4/84	4	4J	5.2 to 6.2
"	5/84	15	8F: 4M: 3J	14.9 to 20.3
N.T.				
Tasman Pt	21/ 4/86	20	3F: 7M	13.5 to 14.8
W.A.				
Rottnest Is	27/ 8/85	10	4F: 1M: 4J	11.5 to 15.2

TABLE 2.6 : Sample Collection Data For Trumpeter Whiting, *Sillago maculata*.

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
N.S.W.				
Sydney	19/ 9/83	20	-	19.0 to 23.4
"	11/ 4/84	2	2F	18.5 to 20.7
"	11/ 5/84	20	13F: 7M	17.5 to 22.1
"	19/ 6/84	16	8F: 5M: 3J	17.6 to 23.9
N.T.				
Tasman Pt	21/ 4/86	20	5F: 4M: 1J	13.5 to 17.4
W.A.				
Dampier	5/ 1/84	12	12J	2.5 to 9.5
Mandurah	12/ 3/86	29	16F:10M: 3J	12.0 to 18.0
NW Shelf	2/ 8/83	15	-	12.1 to 24.7
"	30/ 8/83	6	-	10.8 to 16.1

TABLE 2.7 : Sample Collection Data For Sand Whiting, *Sillago ciliata*.

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
N.S.W.				
Wallis Lake	2/84	10	-	-
Smith's Lake	6/ 7/83	6	-	-
"	1/ 9/83	16	-	8.7 to 15.5
"	2/84	10	-	-
"	2/85	20	-	-
Sydney	6/ 7/83	6	-	-
"	21/ 2/84	8	-	-
"	5/84	3	2F: 1M	20.3 to 22.6

TABLE 2.8 : Sample Collection Data For Golden-Lined Whiting, *Sillago analis.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
N.T.				
Escape Cliffs	22/ 8/84	21	11F: 7M: 3J	12.9 to 20.8
QLD				
Deception Bay	22/ 7/86	19	1F: 2M:16J	9.3 to 26.4
W.A.				
No Name Bay	7/ 1/84	3+	-	10.0 to 16.7

TABLE 2.9 : Sample Collection Data For Yellow Fin Whiting, *Sillago schomburgkii.*

COLLECTION SITE	DATE	NO. OF ANIMALS	SEX RATIO	SIZE RANGE (SL cm)
S.A.				
Angus Inlet	13/ 8/84	21	1F: - :20J	10.3 to 15.9
Spencer Gulf	2/11/85	31	2F:28M	18.1 to 25.7
W.A.				
Dampier	7/ 1/84	7	-	9.9 to 27.8
Sorento	13/ 2/85	1	-	-

APPENDIX 3 : ENZYMES STUDIED, TISSUES INVESTIGATED, ELECTROPHESIS RUNNING CONDITIONS AND PRESUMED NUMBER OF LOCI FOR WHITING SPECIES.

Key: EC= Enzyme Commission number; L= liver, M= muscle, H= heart, E= eye; *= best tissue for this enzyme; 1= TBE pH9, 2= ST EST, 3= TG pH8.5, 4= POULIK, 5= TEB pH 7.8/$MgCl_2$, 6= TM pH7.8, 7= TEM pH7.4, 8= CAEA pH7.2, 9= $CITPO_4$, 10= CAM pH6.1, 11= TC pH5.8; * = best buffer system for this enzyme; AG= agarose, ST= starch, CELL= cellogel, TIT= Helena Titan III plates, ^= best support matrix for this enzyme; A= anodal migration, C= cathodal migration, NS= no staining, P= polymorphic
Note: Loci are designated from the most cathodal in ascending order to the most anodal. e.g. PGM-1 is cathodal of PGM-2 (the faster migrating locus).

TABLE 3.1 : Enzymes Investigated In Whiting Species.

ENZYME	ABBREVIATION	ENZYME COMMISSION NO.
Acid phosphatase	ACPH	EC 3.1.3.2
Aconitase	ACON	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
Alkaline phosphatase	ALKPH	EC 3.1.3.1
D-Amino acid oxidase	DAMOX	EC 1.4.3.3
Aspartate aminotransferase	AAT	EC 2.6.1.1
D-Aspartate oxidase	DASOX	EC 1.4.3.1
Carbonic anhydrase	CA	EC 4.2.1.1
Catalase	CAT	EC 1.11.1.6
Creatine kinase	CK	EC 2.7.3.2
Diaphorase	DIA	EC 1.8.1.4
Enolase	ENOL	EC 4.2.1.11
Esterase	EST	EC 3.1.1.1
Fructose-bisphosphatase	FDP	EC 3.1.3.11
Fumarase	FUM	EC 4.2.1.2
Galactose dehydrogenase	GALDH	EC 1.1.1.48
alpha-Galactosidase	alpha-GAL	EC 3.2.1.22
beta-Galactosidase	beta-GAL	EC 3.2.1.23

TABLE 3.1 (Cont.)

Gluconate dehydrogenase	GDH	EC 1.1.1.69
Glucose-6-phosphate dehydrogenase	G6PDH	EC 1.1.1.49
Glucosephosphate isomerase	GPI	EC 5.3.1.9
alpha-Glucosidase	alpha-GLU	EC 3.2.1.20
beta-Glucuronidase	beta-GUS	EC 3.2.1.31
Glutamate dehydrogenase	GLUD	EC 1.4.1.3
Glutamate-pyruvate transaminase	GPT	EC 2.6.1.2
Glyceraldehyde-3-phosphate dehydrogenase	GA3PDH	EC 1.2.1.12
Glycerol dehydrogenase	GLYDH	EC 1.1.1.6
alpha-Glycerophosphate dehydrogenase	alpha-GPD	EC 1.1.1.8
Glycolate oxidase	GOX	EC 1.1.3.15
Glyoxylase I	GLOI	EC 4.4.1.5
Guanine deaminase	GDA	EC 3.5.4.3
Guanylate kinase	GUK	EC 2.7.4.8
Hexokinase	HK	EC 2.7.1.1
Hexosaminidase	HEX	EC 3.2.1.52
Hydroxyacyl coenzyme A dehydrogenase	HADH	EC 1.1.1.35
beta-Hydroxybutyrate dehydrogenase	HBDH	EC 1.1.1.30
Isocitrate dehydrogenase	IDH	EC 1.1.1.42
Lactate dehydrogenase	LDH	EC 1.1.1.27
Leucine aminopeptidase	LAP	EC 3.4.11.1
Malate dehydrogenase	MDH	EC 1.1.1.37
Malic enzyme	ME	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 3.4.13.9
6-Phosphogluconate dehydrogenase	PGD	EC 1.1.1.44
Pyruvate kinase	PK	EC 2.7.1.40
Sorbitol dehydrogenase	SDH	EC 1.1.1.14
Succinate dehydrogenase	SUCDH	EC 1.3.99.1
Superoxide dismutase	SOD	EC 1.15.1.1
Xanthine dehydrogenase	XDH	EC 1.1.1.204

A total of 55 enzyme systems investigated.

TABLE 3.2 : Electrophoresis of King George Whiting, *Sillaginodes punctata*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4	ST	2A		AAT-1 POOR RESOLUTION
ACON	L	4	ST	1A		
ACPH	L	11	ST	NS		
ADA	L	6	ST	1A		
ADH	L	6	ST	1C		
AK	L	11	ST	3A		VARIABLE IN L
	M	11	ST	1A		
ALD	L	6	ST	2A		POOR ACTIVITY IN ALD-2
ALKPH	L	4	ST	1A		POOR ACTIVITY AND RESOLUTION
CAT	L	4	ST	1A		POOR RESOLUTION
CDA	L	4	ST	NS		
CK	M	4	ST	NS		
DAMOX	L	6	ST	1C	?P	CLOSE TO ORIGIN
	M	6	ST	1C		BEST IN L
DIA	L	6	ST	1A,1C	P	DIA-2
	M	6	ST	NS		
ENOL	L	4	ST	1A		
EST	L	4	ST	2A		BEST ACTIVITY
	M	4	ST	1A		IN L
FUM	L	4	ST	1A		
GAPDH	M	4	ST	1A		POOR ACTIVITY, ON ORIGIN
GDA	L	4	ST	NS		

TABLE 3.2 (cont.)

ENZYME		TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
GLYDH		L	4	ST	NS		
GOX		L	4	ST	1A		
GPD		M	4	ST	1A		POOR ACTIVITY
GPI		L	4	ST	1A		
		M[D]	4	ST	3A	P	GPI-1,2,3 IN M
GPT		L	4	ST	1A	P	
HEX		L	11	ST	1A		POOR RESOLUTION
HK		L	4	ST	1A		
IDH		L	4,11*	ST	1A		POOR ACTIVITY
		M	4,11*	ST	1A		IN M
LAP		L	6	ST	1A		POOR RESOLUTION
LDH		L	4	ST	1A		
MDH		M	11	ST	2A		POOR RESOLUTION
ME		L[W]	11	ST	2A		VARIABLE IN L
		M[W]	11*	ST,AG	1A	?P	POOR RESOLUTION IN AGAROSE
MPI		L	4	ST	1A		
		M[D]	4	ST	1A		
PEP	(FP)	L[C],M	4	ST	1A		VARIABLE
	(LY)	L,M[C]	4	ST	2A		BETTER IN M
	(PL)	L	6	ST	2A		
PGD		L[D]	6	ST	1A	P	BEST ACTIVITY
		M[D]	6	ST	1A	P	IN L
PGM		L	6	ST	2A		POOR RESOLUTION
		M	6	ST,AG	1A		POOR RESOLUTION IN AGAROSE
PK		L	4	ST	NS		
SDH		L	4	ST	1A		
SOD		L	4	ST	1A		
XDH		L	4	ST	1A		

38 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 46 PRESUMED LOCI. 10 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.3 : Electrophoresis of School Whiting,
Sillago bassensis bassensis

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4,10*	ST	2A,2C	?P	AAT-1 POOR ACTIVITY
ADA	1	11	ST	1A		POOR RESOLUTION AND ACTIVITY
ADH	L	4,6*,10	ST	1C	?P	VARIABLE
AK	L	6	ST	2A		POOR RESOLUTION
	M	6	ST	1A		
ALD	M	6	ST	1A		POOR ACTIVITY
CAT	L	4	ST	1A	?P	
CDA	M	10	ST	3A,1C		POOR ACTIVITY
DIA	L	4	ST	1A	?P	VARIABLE
EST	L	4	ST	1A	?P	
FUM	M	11	ST	1A		
GDA	M	6	ST	1A		POOR ACTIVITY
GOX	L	4	ST	1A		POOR ACTIVITY
G6PDH	L	4	ST	NS		
GPI	L	4	ST	1A		GPI-1 M,P
	M	4	ST	3A	P	GPI-2 M,P GPI-3 M,P GPI-4 L
GPT	L	4	ST	1A		
HEX	L	4	ST	NS		
IDH	L	10,11*	ST	1A		IDH-1 M
	M	10,11*	ST	2A		IDH-2 L, M
LDH	M	4	ST	1A		
MDH	L	11	ST	1A		
	M	10,11*		2A		
ME	L	11	ST	1A		
	M	11	ST	1A		
MPI	M	4	ST	1A		

TABLE 3.3 (cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
PEP (LGG)	L	6	ST	3A	?P	AS FOR PL
(PL)	L	6	ST	2A	?P	
(FP)	L	6	ST	1A		VARIABLE
PGD	L	6,10*	ST	1A		
PGM	L	6	ST	2A	P	PGM-1
SDH	L	4	ST	1A		GOOD ACTIVITY
SOD	L	4,11*	ST	1A		GOOD ACTIVITY
XDH	L	4	ST	1A		POOR ACTIVITY

27 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 40 PRESUMED NUMBER OF LOCI. 10 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.4 : Electrophoresis of Red Spot Whiting, *Sillago bassensis flindersi*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4*,8,10	ST^,TIT	2A	?P	AAT-1 POOR
	M	4	ST	2A		RESOLUTION
	H	4	"	2A		
	E	4	"	NS		AAT-2 L,H BEST
ACON	L	7	ST	2A		ACON-1 L,M,H
	M	7	"	1A		ACON-2 H
	H	7	"	2A		ACON-3 L
	E	7	"	NS		
ADA	L	8,10*,11	ST	1A		POOR RESOLUTION
	M	10,11*	ST^,TIT	1A	?P	BEST ACTIVITY
	H	11	ST	1A		IN M
	E	11	"	1A		
ADH	L	8,10*,11	ST^,TIT,CEL	1C	P	ADH-1 L
	M	11	"	1A,1C		
	H	11	"	1C		
	E	11	"	1C		
AK	L	8,10*,11	ST	2A		AK-1 L,M,H,E
	M	10*,11	ST^,TIT	2A		AK-2 H
	H	11	ST	2A		AK-3 E
	E	11	"	2A		
ALD	L	7	ST	1A		ALD-1 M
	M	7,8,10*	ST^,TIT	1A	?P	ALD-2 L
	H	7	ST	NS		ALD-3 E
	E	7	"	1A		POOR ACTIVITY
						IN L
CAT	L	4,8,10*	ST	1A	?P	POOR RESOLUTION
	M	10	"	1A		
	H	10	"	1A		
	E	10	"	1A		
DAMOX	L	6,8,9,10*	ST,TIT^	1A	?P	POOR RESOLUTION
	M	6	ST	NS		
DASOX	L	10	ST	1C		DASOX-2 IN M,
	M	10	"	1A,1C		POOR ACTIVITY,
	H	10	"	NS		SAME AS DAMOX
	E	10	"	NS		
DIA	L	1*,6,8,10	ST^,TIT,CEL	2A		DIA-1 L; POOR
	M	1	ST	1A		RESOLUTION
	H	1	"	1A		DIA-2 L,E
	E	1	"	1A		DIA-3 M,H
						POOR ACTIVITY
						IN ALL TISSUES

TABLE 3.4 (cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
ENOL	L	7	ST	1A		BEST ACTIVITY
	M	7	"	1A		IN M
	H	7	"	1A		
	E	7	"	NS		
EST	L	4*,6,8,10	ST	2A	?P	EST-2
	M	4	"	2A		BEST ACTIVITY
	H	4	"	2A		IN L
	E	4	"	1A		
FDP	L	6	ST	1A		
FUM	L	7	ST	1A		
	M	7	"	1A		
	H	7	"	1A		
	E	7	"	1A		
alpha-GAL	L	6	ST	1A		POOR ACTIVITY
beta-GAL	L	6	ST	1A		STREAKS
GDA	L	1	ST	1A		GDA-1 M
	M	1	"	2A		GDA-2 L,M,H
	H	1	"	1A		VARIABLE
	E	1	"	NS		
GAL-6-PDH	L	1	ST	1A,1C		POOR ACTIVITY
	M	1	"	1A		IN ALL TISSUES
	H	1	"	1A		
	E	1	"	2C		
GLYDH	L	4	ST	1A		
GOX	L	3,8,10*	ST^,TIT	1A		POOR ACTIVITY
	M	3,8,10*	"	1A		
alpha-GPD	L	6	ST	1A		GPD-1 M
	M	6	"	1A		GPD-2 L,H
	H	6	"	1A		BEST ACTIVITY
	E	6	"	NS		IN M
G6PDH	L	4*,10	ST,TIT	1A		BEST ACTIVITY
	M	4*,10	ST	1A		IN E
	H	4*,10	"	2A		VARIABLE
	E	4*,10	"	1A		
GPI	L	4*,8,9,10	ST,TIT	1A		GPI-1 M,H,E
	M	4*,8,9,10	ST	3A	P	GPI-2 M,H,E
	H	4*,8,9,10	"	3A		GPI-3 L,M,H,E
	E	4*,8,9,10	"	3A		
GPT	L	4	ST	1A		POOR ACTIVITY
	M	4	"	NS		

TABLE 3.4 (cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
beta-GUS	L	6*,7	ST	2A		BEST ACTIVITY
	M	6	"	NS		IN H
	H	6	"	1A		POOR RESOLUTION
	E	6	"	NS		
HADH	L	3	TIT	1A		POOR ACTIVITY
HBDH	L	4	ST	1A		BEST ACTIVITY
	M	4	"	1A		IN M
HEX	L	11	ST	1A		BEST ACTIVITY
	M	11	"	1A		IN L
	H	11	"	1A		POOR RESOLUTION
	E	11	"	NS		
HK	L	4*,8,10	ST	1A		BEST ACTIVITY
	M	4	"	3A		IN E
	H	4	"	2A		
	E	4	"	1A		
IDH	L[cm]	8,10*,11	ST,TIT	1A	?P	IDH-1 M
	M	8,10*,11	"	1A	?P	IDH-2 L
	H	8	ST	1A		BEST ACTIVITY
	E	8	"	NS		IN H
						E ?NULL ALLELE
LAP	L	10	ST	1A		POOR ACTIVITY
LDH	L	1*,6	ST^,CELL	1A		TISSUE DIFF.S
	M	1	"	1A		
	H	1	"	1A		SUB BANDS
	E	1	"	1A		ANODALLY
MDH	L	6,11*	ST,TIT,CEL	1A		POOR RESOLUTION
	M	6,11*	"	1A		IN L
	H	11	ST	2A		BEST ACTIVITY
	E	11	"	1A		IN H
ME	L[cm]	10*,11	ST,TIT^	1A		ME-1 M,H
	M[cm]	10*,11	ST	2A	?P	ME-2 H,E
	H	11	"	2A		POOR ACTIVITY
	E	11	"	1A		IN L
						E ?NULL ALLELE
MPI	L	4*,8,10	ST,TIT	1A	P	BEST ACTIVITY
	M[cm]	4*,8,10	"	1A	P	IN M & H
	H	4	ST	1A	P	ANODAL SUB-
	E	4	"	1A	P	BANDING ON TIT
NP	L	3	TIT	1A		COMPLEX PATTERN

TABLE 3.4 (cont.)

ENZYME		TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
PEP	(VA)	L	8,10*	ST	1C		POOR RESOLUTION
	(L66)	L	8,10*	"	2A,1C	P	BETTER RESOLUTION
	(PL)	L	8,10*	ST^,TIT	1A,1C	P	"
	(FP)	L	8,10*	"	2A,1C	?P	POOR RESOLUTION
	(LAP)	L[c.]	8,10*	ST	1A		POOR ACTIVITY
	(LLL)	L	8	"	3A,1C		"
PGD		L[c.]	6,7,8,10*	ST^,TIT,CEL	1A	P	BEST ACTIVITY
		M	6	ST	1A		IN L & E
		H	6	"	1A		VARIABLE
		E	6	"	1A		
PGM		L	6*,8,10	ST,TIT,CEL	2A	P	PGM-1 L,M,H
		M	6	ST,TIT	1A	P	PGM-2 L,H
		H	6	ST	2A		? NULL ALLELE
		E	6	"	NS		
PK		L	1	ST	NS		PK-1 M
		M	1	"	2A		PK-2 H
		H	1	"	1A		PK-3 M
		E	1	"	NS		
SDH		L	4	ST	2A		BEST ACTIVITY
		M	4	"	2A		IN L
		H	4	"	1A		VARIABLE
		E	4	"	1A		
SOD		L	4*,6,8	ST	1A		
		M	4*,6,8	"	1A		
SUCDH		L	4	ST	1A		POOR ACTIVITY
		M	4	"	1A		
XDH		L	4	ST	1A		XDH-1 H,E
		M	4	"	1A		XDH-2 L,M
		H	4	"	1A		BEST ACTIVITY
		E	4	"	1A		IN L
							VARIABLE

44 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 75 PRESUMED NUMBER OF LOCI. 16 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.5 : Electrophoresis of Banded Whiting, *Sillago vittata*.

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4,10*	ST	2A,2C		AAT-1 C. POOR ACTIVITY & RESOLUTION AAT-2A
ADA	1	11	ST	1A		GOOD ACTIVITY
ADH	L	4,6*,10	ST	1C		POOR ACTIVITY
AK	L	6	ST	2A		POOR RESOLUTION IN L
	M	6	ST	1A		GOOD ACTIVITY
ALD	M	6	ST	1A	?P	POOR ACTIVITY
CAT	L	4	ST	1A	?P	POOR ACTIVITY
CDA	M	10	ST	4A,4C		POOR ACTIVITY
DIA	L	4	ST	2A		DIA-2 POOR ACTIVITY
EST	L	4	ST	1A		
FUM	M	11	ST	1A		POOR ACTIVITY & RESOLUTION
GDA	M	6	ST	1A	?P	POOR ACTIVITY
GOX	L	4	ST	NS		
G6PDH	L	4	ST	NS		
GPI	L	4	ST	NS		
	M	4	ST	3A	P	GPI-2 & 3
GPT	L	4	ST	1A		POOR ACTIVITY
HEX	L	4	ST	NS		
IDH	L	10,11*	ST	1A	?P	POOR ACTIVITY IDH-2L
	M	10,11*	ST	2A		GOOD ACTIVITY IDH-1
LDH	M	4	ST	1A		GOOD ACTIVITY
MDH	L	11	ST	1A		MDH-1, L,M
	M	10,11*		2A		MDH-2 M
ME	L	11	ST	1A		POOR ACTIVITY ME-2 L
	M	11	ST	2A		ME-1 M
MPI	M	4	ST	1A	P	

TABLE 3.5 (cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
PEP (LGG)	L	6	ST	2A		POOR RESOLUTION
(PL)	L	6	ST	2A		
(FP)	L	6	ST	1A		VARIABLE
PGD	L	6,10*	ST	1A	?P	
PGM	L	6	ST	2A		PGM-1
SDH	L	4	ST	1A	?P	POOR ACTIVITY
SOD	L	4*,11	ST	1A		GOOD ACTIVITY AND RESOLUTION
XDH	L	4	ST	1A		POOR ACTIVITY

27 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 44 PRESUMED NUMBER OF LOCI. 9 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.6 : Electrophoresis of Stout Whiting, *Sillago robusta*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI	COMMENTS
AAT	L	4*,6	ST	2A	AAT-1 L,E
	M	4	ST	1A	STREAKY IN L
	H	4	ST	1A	AAT-2 L,H
	E	4	ST	1A	
ACON	L	11	ST	2A	
	M	11	ST	NS	
	H	11	ST	NS	
	E	11	ST	NS	
ACPH	L	1,10*	ST	1A	BEST ACTIVITY
	M	1*,10	ST	2A	FAINT
	H	1*,10	ST	NS	
	E	1*,10	ST	1A	FAINT
ADA	L	10	ST	1A	BEST ACTIVITY
	M	10	ST	1A	
	H	10	ST	1A	FAINT
	E	10	ST	1A	
ADH	L	6	ST	1C	
AK	L	11,10*	ST	2A	FAINT
	M	11,10*	ST	1A	
	H	11,10*	ST	1A	
	E	11,10*	ST	1A	
ALD	L	10*,7	ST	1A,1C	NEAR ORIGIN
	M	10*,7	ST	1C	
	H	10*,7	ST	NS	
	E	10*,7	ST	NS	
CAT	L	4	ST	1A	
	M	4	ST	NS	
	H	4	ST	NS	
	E	4	ST	NS	
DAMOX	L	11	ST	1A,1C	NEAR ORIGIN
	M	11	ST	1C	POOR ACTIVITY
	H	11	ST	NS	
	E	11	ST	NS	
DASOX	L	11	ST	1A,1C	POOR ACTIVITY
	M	11	ST	1C	
DIA	L	6	ST	1A	
	M	6	ST	1A	FAINT,
	H	6	ST	NS	TISSUE
	E	6	ST	1A	DIFFERENCES
ENOL	L	7	ST	1A	BEST
	M	7	ST	1A	ACTIVITY
	H	7	ST	1A	IN M
	E	7	ST	1A	

TABLE 3.6 (Cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
EST	L	6	ST	1A		POOR ACTIVITY
	M	6	ST	2A		IN ALL TISSUES
	H	6	ST	NS		
	E	6	ST	2A		
FUM	L	10	ST	1A		
	M	10	ST	NS		
	H	10	ST	NS		
	E	10	ST	NS		
GDA	L	1*,4	ST	1A		GDA-2
	M	1*,10	ST	2A	?P	GDA-2 M
	H	1	ST	2A		TISSUE
	E	1	ST	1A		DIFFERENCES
GA-3-PDH	L	10*,7	ST	NS		
	M	10*,7	ST	1A		
	H	10*,7	ST	1A		
	E	10*,7	ST	2A		
GDH	L	1	ST	NS		
	M	1	ST	1A		
	H	1	ST	NS		
	E	1	ST	1A		STREAKY
GAL-6-PDH	L	1	ST	2A		POOR ACTIVITY
	M	1	ST	1A		
	H	1	ST	2A		
	E	1	ST	NS		
GLO-1	L	10	ST	NS		
	M	10	ST	1A		POOR ACTIVITY
	H	10	ST	1A		STREAKY
	E	10	ST	1A		ST
G6PDH	L	1*,4	ST	2A	?P	G6PDH-1 L
	M	1*,4	ST	1A		G6PDH-2 M,H
	H	1*,4	ST	1A		G6PDH-3 L,E
	E	1*,4	ST	1A		
alpha-GPD	L	4,10*	ST	1A		NEAR ORIGIN
	M	4,10*	ST	1A		POOR RESOLUTION
GPI	L	4	ST	1A		BEST IN M
	M	4	ST	3A	P	GPI-1 M,E
	H	4	ST	1A		GPI-2 M,E
	E	4	ST	3A		GPI-3 L,M,H,E
GPT	L	4	ST	1A		BEST
	M	4	ST	1A		ACTIVITY
	H	4	ST	NS		IN L
	E	4	ST	NS		
GUK	L	7	ST	NS		POOR RESOLUTION
	M	7	ST	NS		
	H	7	ST	NS		
	E	7	ST	1A		

TABLE 3.6 (Cont.)

ENZYME		TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
beta-GUS		L	7	ST	1A		beta-GUS-1 M,H
		M	7	ST	1A		beta-GUS-2 L,E
		H	7	ST	2A		
		E	7	ST	1A		POOR ACTIVITY
HBDH		L	4	ST	1A		HBDH-1 H
		M	4	ST	NS		HBDH-2 L,E
		H	4	ST	1A		POOR ACTIVITY
		E	4	ST	1A		
HEX		L	10*,11	ST	1A		SINGLE LOCUS
		M	10*,11	ST	NS		BEST
		H	10*,11	ST	1A		ACTIVITY
		E	10*,10	ST	NS		IN L
HK		L	4,7	ST	NS		
		M	4,7	ST	NS		
		H	4,7	ST	NS		
		E	4,7	ST	NS		
IDH		L	4,10,11*	ST	1A	P	IDH-1 M,H,E
		M	4,10,11*	ST	2A	P	IDH-2 L,M
		H	10	ST	1A		
		E	10	ST	1A		
LAP		L	10	ST	1A		POOR ACTIVITY
		M	10	ST	1A		
		H	10	ST	1A		
		E	10	ST	NS		
LDH		L	1	ST	1A		LDH-1 L,E
		M	1	ST	1A		LDH-2 M,H,E
		H	1	ST	1A		LDH-3 E
		E	1	ST	3A		
MDH		L	10,11*	ST	2A		GOOD ACTIVITY
		M	10,11*	ST	2A		POOR
		H	10,11*	ST	2A		SEPARATION
		E	10,11*	ST	2A		OF LOCI
ME		L	4,11*	ST	1A	?P	VARIABLE
		M	4,11*	ST	2A		
		H	11	ST	2A		
		E	11	ST	NS		
MPI		L	4	ST	1A	P	BEST
		M	4	ST	1A	P	ACTIVITY
		H	4	ST	1A	P	IN E
		E	4	ST	1A	P	
PEP	(FP)	L,M,H,E	4	ST	1A		BEST IN L
	(LGG)	L	4	ST	2A		VARIABLE
	(LY)	L[e],M	4	ST	2A		
	(VL)	L	4	ST	2A		

TABLE 3.6 (Cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
PGD	L	6	ST	1A		VARIABLE
	M	6	ST	NS		
	H	6	ST	NS		
	E	6	ST	NS		
PGM	L	6	ST	3A	P	PGM-1 L,M,E
	M	6	ST	1A	P	PGM-2 L
	H	6	ST	NS		PGM-3 L,E
	E	6	ST	2A		
PK	L	1	ST	NS		BEST
	M	1	ST	1A		ACTIVITY
	H	1	ST	2A		IN M
	E	1	ST	2A		
SDH	L	4*,7,10	ST	1A	P	
	M	4*,7	ST	NS		POOR ACTIVITY
	H	4*,7	ST	NS		IN M
	E	4*,7	ST	NS		
SOD	L	4	ST	3A		
	M	4	ST	1A		
XDH	L	4,7*	ST	NS		XDH-1 M
	M	4,7*	ST	1A		XDH-2 H,E
	H	4,7*	ST	1A		BEST ACTIVITY
	E	4,7*	ST	1A		IN M & H

42 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 65 PRESUMED NUMBER OF LOCI. 12 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.7 : Electrophoresis of Trumpeter Whiting, *Sillago maculata maculata*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4	ST	1A	P	AAT-3 L
	M	4	ST	NS		
	H	4	ST	2A		AAT-1,2 H
	E	4	ST	NS		
ACON	L	9	ST	2A		
	M	9	ST	NS		
	H	9	ST	NS		
	E	9	ST	NS		
ACPH	L	1	ST	2A		
	M	1	ST	2A		
	H	1	ST	1A		STREAKY
	E	1	ST	2A		STREAKY
ADA	L	11	ST	1A		BEST RESOLUTION
	M	11	ST	1A		IN M
ADH	L	1,4*	ST	1C		
	M	1,4*	ST	NS		
	H	1	ST	NS		
	E	1	ST	NS		
AK	L	9*,11	ST	2A		BEST
	M	9*,11	ST	1A		ACTIVITY
	H	9*,11	ST	1A		IN M & H
	E	9*,11	ST	1A		
ALD	L	9*,7	ST	1A,1C		BEST
	M	9*,7	ST	1A,1C		ACTIVITY
	H	9*	ST	1C		IN H & E
	E	9*	ST	1A		
CAT	L	4	ST	1A	?P	BEST ACTIVITY L
	M	4	ST	NS		
	H	4	ST	1A		BEST RESOLUTION H
	E	4	ST	NS		
DAMOX	L	11	ST	1A		STREAKY
	M	11	ST	1A		POOR ACTIVITY, ON
	H	11	ST	NS		ORIGIN
	E	11	ST	NS		
DASOX	L	11	ST	1C		POOR ACTIVITY
	M	11	ST	1C		POOR ACTIVITY
DIA	L	6	ST	1A		DIA-1 M,H,E
	M	6	ST	1A		DIA-2 L
	H	6	ST	1A		M,H,E POOR ACTIVITY
	E	6	ST	1A		

TABLE 3.7 (Cont.)

ENOL	L	7	ST	1A		
	M	7	ST	1A		
	H	7	ST	1A		
	E	7	ST	2A	?P	SLOW LOCUS
EST	L	6	ST	1A		BEST ACTIVITY
	M	6	ST	2A		IN L
	H	6	ST	2A		TISSUE
	E	6	ST	2A		DIFFERENCES
FUM	L	9	ST	1A		POOR ACTIVITY
	M	9	ST	NS		
	H	9	ST	NS		
	E	9	ST	1A		
alpha-GAL	L	6	ST	1A		STREAKY
beta-GAL	L	6	ST	1A		STREAKY
	M	6	ST	1A		
GDA	L	1	ST	1A		POOR ACTIVITY
	M	1	ST	2A		
	H	1	ST	2A		
	E	1	ST	1A		
GOX	L	4	ST	1A		
	M	4	ST	NS		
GA-3-PDH	L	9	ST	1A		POOR RESOLUTION
	M	9	ST	1A		IN L
	H	9	ST	1A		
	E	9	ST	2A		
G6PDH	L	4	ST	1A		STREAKY
	M	4	ST	NS		
	H	4	ST	NS		
	E	4	ST	1A		BETTER RESOLUTION
GPI	L	4	ST	1A		
	M	4	ST	3A	?P	GPI-2,3
	H	4	ST	3A	?P	GPI-1 M,H
	E	4	ST	3A		
GPT	L	4	ST	1A		BEST
	M	4	ST	1A		ACTIVITY
	H	4	ST	NS		IN L
	E	4	ST	NS		

TABLE 3.7 (Cont.)

GUK	L	7	ST	NS		
	M	7	ST	NS		
	H	7	ST	NS		
	E	7	ST	1A		
beta-GUS	L	6*,7	ST	1A		POOR RESOLUTION
	M	6*,7	ST	1A		POOR ACTIVITY
	H	6*,7	ST	2A		
	E	6*,7	ST	NS		
HBDH	L	4	ST	1A		L/E LOCUS
	M	4	ST	NS		
	H	4	ST	1A		POOR ACTIVITY LOCUS
	E	4	ST	1A		BETTER RESOLUTION
HK	L	4*,7	ST	1A		POOR ACTIVITY
	M	4*,7	ST	1A		
	H	4*,7	ST	1A		TISSUE
	E	4*,7	ST	1A		DIFFERENCES
IDH	L	11	ST	1A	P	IDH-2 L
	M	11	ST	2A	P	IDH-1 M
	H	11	ST	2A		
	E	11	ST	NS		
LDH	L	1	ST	1A		LDH-1 H,E
	M	1	ST	1A		LDH-2 M,L,E
	H	1	ST	1A		LDH-3 E
	E	1	ST	3A		
MDH	L	9,11*	ST	1A		MDH-1 M,H,E
	M	9,11*	ST	2A	?P	MDH-1 IN H
	H	9,11*	ST	2A		
	E	9,11*	ST	2A		MDH-2 M,H,E,L
ME	L	11	ST	1A		VARIABLE
	M	11	ST	1A		
	H	11	ST	2A		
	E	11	ST	NS		
MPI	L	4	ST	1A	P	BEST
	M	4	ST	1A		ACTIVITY
	H	4	ST	1A		IN H
	E	4	ST	1A		
PEP (FP)	L^c,M,H^c,E	4	ST	1A		VARIABLE
(LY)	L^c,M	4	ST	3A		
(VL)	L,M	6	ST	1A		
PGD	L	6	ST	1A	P	VARIABLE
	M	6	ST	1A		POOR ACTIVITY
	H	6	ST	1A		
	E	6	ST	1A		

TABLE 3.7 (Cont.)

PGM	L	6	ST	2A	?P	PGM-1 IN L
	M	6	ST	1A		
	H	6	ST	1A		
	E	6	ST	2A		
PK	L	1	ST	1A		BEST
	M	1	ST	3A		ACTIVITY
	H	1	ST	1A		IN M
	E	1	ST	1A		
SDH	L	4*,7	ST	1A	?P	
	M	4*,7	ST	1A		TISSUE
	H	4*,7	ST	NS		DIFFERENCES
	E	4*,7	ST	NS		
SOD	L	4	ST	1A		
	M	4	ST	1A		
SUCDH	L	4	ST	1A		BEST ACTIVITY
	M	4	ST	1A		IN L
XDH	L	4,7*	ST	1A		BEST ACTIVITY
	M	4,7*	ST	1A		IN M,H
	H	4,7*	ST	1A		TISSUE
	E	4,7*	ST	1A		DIFFERENCES

39 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 60 PRESUMED NUMBER OF LOCI, 13 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.8 : Electrophoresis of Western Trumpeter Whiting, *S. maculata burrus*.

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	M	10	ST	1A	?P	
ADA	L	4	ST	1A		ON BORATE FRONT
ADH	L	10	ST	1C	?P	
EST	L	4	ST	1A		
GDA	L	4	ST	1A		
GPI	L	4	ST	1A	P	GPI-1,2,3 IN M
	M	4	"	3A	?P	GPI-4 IN L
IDH	L	10	ST	1A		
	M	10	ST	1A	P	Idh-2 M
MDH	M	10	ST	2A		
ME	L	10	ST	1A	?P	
MPI	M	4	ST	1A	P	
PEP (FP)	L	4	ST	1A		VARIABLE
(PL)	L	10	ST	1A		
PGD	L	10	ST	1A		
PGM	L	4	ST	2A	P	Pgm-2
	M	6	ST	1A	P	Pgm-1
SDH	L	4	ST	2A		
	M	4	ST	NS		
SOD	L	10	ST	1A		
XDH	M	4	ST	NS		

18 ENZYMES INVESTIGATED, REPRESENTING 23 PRESUMED NUMBER OF LOCI, 11 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.9 : Electrophoresis of Sand Whiting, *Sillago ciliata*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4*,7	ST	2A		AAT-1 POOR
	M	4*,7	ST	2A	P	AAT-2 M,H
	H	4	ST	2A		AAT-3 L
	E	4	ST	NS		
ACON	L	4,7,11*	ST	2A		ACON-1 H
	M	4,7,11*	ST	1A		ACON-2 L,M
	H	11	ST	1A		ACON-3 L
	E	11	ST	NS		
ACPH	L	1*,2,9	ST	2A		
	M	1*,2,9	ST	2A		
	H	9	ST	1A		STREAKY
	E	9	ST	NS		
ADA	L	9	ST	1A		NO TISSUE
	M	9	ST	1A		DIFFERENCES
	H	9	ST	1A		
	E	9	ST	1A		
ADH	L	1*,9	ST	1A		BEST
	M	1*,9	ST	1A		ACTIVITY
	H	1	ST	NS		IN L
	E	1	ST	NS		
AK	L	11,9*	ST	2A		BEST
	M	11,9*	ST	1A		ACTIVITY
	H	11,9*	ST	1A		IN M
	E	11,9*	ST	1A		
ALD	L	6,9*	ST	2A	?P	COMPLEX
	M	6,9*	ST	4A	?P	PATTERN
	H	9	ST	NS		
	E	9	ST	NS		
ARS	L	9	ST	NS		
	M	9	ST	NS		
CA	L	4	ST	1A		FAINT
	M	4	ST	1A		
CAT	L	4	ST	1A	?P	BEST
	M	4	ST	1A		ACTIVITY
	H	4	ST	NS		IN L
	E	4	ST	NS		
CK	L	1	ST	NS		
	M	1	ST	NS		
DAMOX	L	9*,11	ST	1A		
	M	9*,11	ST	1C		SAME AS
	H	11	ST	NS		DASOX
	E	11	ST	NS		

TABLE 3.9 (Cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
DASOX	L	9	ST	NS		
	M	9	ST	1C		
DIA	L	6	ST	2A	P	DIA-1 L,E
	M	6	ST	NS		DIA-2 H,L
	H	6	ST	1A		
	E	6	ST	2A		
ENOL	L	7	ST	1A	P	
	M	7	ST	1A		
	H	7	ST	1A		
	E	7	ST	2A		
EST	L	2,4*,6	ST	3A	P	BEST
	M	2,4*,6	ST	1A		ACTIVITY
	H	6	ST	1A		IN L
	E	6	ST	1A		
FUM	L	6*,9	ST	2A		BEST
	M	6*,9	ST	1A,1C		ACTIVITY
	H	9	ST	NS		IN L
	E	9	ST	NS		
alpha-GAL	L	2	ST	1A		
	M	2	ST	NS		
beta-GAL	L	2	ST	1A	?P	COMPLEX
	M	2	ST	NS		PATTERN
GDA	L	1*,2	ST	1A	P	BEST
	M	1*,2	ST	1A		ACTIVITY
	H	1	ST	NS		IN L
	E	1	ST	NS		
GA-3-PDH	L	7*,9	ST	1A,1C		BEST
	M	7*,9	ST	2A		ACTIVITY
	H	9	ST	1A		IN L
	E	9	ST	2A		
GDH	L	1	ST	NS		
	M	1	ST	NS		
	H	1	ST	NS		
	E	1	ST	1A		STREAKY
GALDH	L	1	ST	2A		GALDH-1 M,H
	M	1	ST	1A		GALDH-2 L,E
	H	1	ST	2A		BEST ACTIVITY
	E	1	ST	1A		IN L
GLO-1	L	9	ST	NS		
	M	9	ST	1A		POOR ACTIVITY
	H	9	ST	1A		STREAKY
	E	9	ST	1A		STREAKY
GOX	L	9	ST	1A	?P	POOR ACTIVITY
	M	9	ST	NS		

TABLE 3.9 (Cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
alpha-GPD	L	7	ST	1A		alpha-GPD- 1 M
	M	7	ST	1A		alpha-GPD- 2 L
						BEST ACTIVITY
						IN M
GLYDH	L	4	ST	1A		GLYDH -1 M
	M	4	ST	1A	?P	GLYDH -2 POOR
						ACTIVITY
G6PDH	L	1*,4	ST	1A	?P	BEST
	M	1*,4	ST	1A		ACTIVITY
	H	1*,4	ST	1A		IN L
	E	1*,4	ST	1A		
GPI	L	4*,6	ST	1A	?P	
	M	4*,6	ST,TIT	3A	P	GPI-3
	H	6	ST	3A		
	E	6	ST	3A		
GPT	L	4	ST	1A	P	
	M	4	ST	NS		
	H	4	ST	NS		
	E	4	ST	NS		
GUK	L	7	ST	NS		
	M	7	ST	NS		
	H	7	ST	NS		
	E	7	ST	1A		
beta-GUS	L	7	ST	1A		POOR ACTIVITY
	M	7	ST	NS		IN ALL TISSUES
	H	7	ST	1A		
	E	7	ST	1A		
HBDH	L	4	ST	1A		
	M	4	ST	2A		POOR ACTIVITY
	H	4	ST	1A		
	E	4	ST	1A		
HEX	L	9*,11	ST	1A		BEST
	M	9*,11	ST	NS		ACTIVITY
	H	9*,11	ST	1A		IN L
	E	9*,9	ST	NS		
HK	L	2,4*,7	ST	1A		HK-1 M
	M	2,4*,7	ST	1A		HK-2 H,L,E
	H	2,4*,7	ST	1A		BEST ACTIVITY
	E	2,4*,7	ST	1A		IN H
IDH	L	7,11*	ST	1A	P	IDH-1 M,H
	M	7,11*	ST	2A		IDH-2 L,E,M
	H	11	ST	1A		
	E	11	ST	1A		

TABLE 3.9 (Cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
LAP	L	4,9*	ST	1A		POOR ACTIVITY
	M	4,9*	ST	1A		IN ALL
	H	9	ST	2A		TISSUES
	E	9	ST	1A		
LDH	L	1	ST	1A		LDH-1 M,H,E
	M	1	ST	1A		LDH-2 H,L
	H	1	ST	2A		LDH-3 E
	E	1	ST	2A		
MDH	L	4,9,11*	ST	1A	?P	MDH-1 L,M
	M	4,9,11*	ST,TIT	2A	?P	
	H	9,11*	ST	2A		
	E	9,11*	ST	NS		
ME	L	4,11*	ST	1A		BEST
	M	4,11*	ST,TIT	2A		ACTIVITY
	H	11	ST	1A		IN L
	E	11	ST	2A		ALL VARIABLE
MPI	L	4	ST	1A	P	
	M	4	ST,TIT	1A		
	H	4	ST	1A		
	E	4	ST	1A		
PEP (AP)	L	4	ST	1A		
(FP)	L[c],H,E	4	ST	1A		
(LGG)	L,M	4	ST	2A		
(LLL)	L	4	ST	2A	?P	
(VA)	L	4	ST	1A		
(LY)	L[c],M	4	ST	3A		
PGD	L	6	ST	1A		VARIABLE
	M	6	ST	1A		POOR ACTIVITY
	H	6	ST	1A		
	E	6	ST	NS		
PGM	L	4,6	ST	2A	P	PGM-1 L,M
	M	4,6	ST,TIT	1A	P	BEST IN M
	H	6	ST	2A		
	E	6	ST	2A		
PK	L	1	ST	1A		BEST
	M	1	ST	1A		ACTIVITY
	H	1	ST	1A		IN M
	E	1	ST	1A		
SDH	L	1*,4,7	ST	1A	?P	
	M	1*,4,7	ST	1A		POOR ACTIVITY
	H	4*,7	ST	NS		
	E	4*,7	ST	NS		

TABLE 3.9 (Cont.)

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
SOD	L	4	ST	1A	P	
	M	4	ST	1A	P	
	H	4	ST	1A		
	E	4	ST	NS		
SUCDH	L	4	ST	1A		POOR ACTIVITY
	M	4	ST	1A		
XDH	L	1,4*,7	ST	1A		BEST
	M	1,4*,7	ST	2A		ACTIVITY
	H	4*,7	ST	1A		IN L
	E	4*,7	ST	1A		

49 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 81 PRESUMED NUMBER OF LOCI, 23 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.10 : Electrophoresis of Golden-Lined Whiting, *S. analis*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4*,10	ST	1A		
GDA	M	4	ST	NS		
GPD	L	4	ST	NS		
GPI	M	4	ST	3A	?P	Gpi-2, Gpi-3
IDH	M	4	ST	NS		
LDH	L	4	ST	2A		
PEP (L66)	L	4	ST	2A		
(FP)	L	4	ST	1A	?P	POOR ACTIVITY
PGD	L	4	ST	1A		POOR ACTIVITY
PGM	L	4	ST	1A	P	
SDH	M	4	ST	NS		
SOD	M	4	ST	1A		

11 ENZYME SYSTEMS INVESTIGATED, REPRESENTING 12 PRESUMED NUMBER OF LOCI, 4 SUSPECTED POLYMORPHIC LOCI.

TABLE 3.11 : Electrophoresis of Yellow Fin Whiting, *Sillago schomburgkii*

ENZYME	TISSUE	BUFFERS	SUPPORT MATRIX	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	4	ST	1A		
AK	M	8,11*	ST	2A	?P	AK-2
CAT	L	4	ST	1A		POOR RESOLUTION
DIA	L	4*,8	ST	2A		GOOD RESOLUTION Dia-2 FAINT
EST	L	4	ST	2A	?P	Est-2
G6PDH	M	4	ST	NS		
GPI	L	8	ST	2A		POOR ACTIVITY
	M	4*,11	ST	3A		
IDH	M	11	ST	2A		Idh-2 FAINT
LDH	M	4	ST	1A		
MDH	L	11	ST	2A		
ME	M	8,11*	ST	1A		VARIABLE
MPI	M	4*,8	ST	1A	P	
PEP (FP)	L	4,8*	ST	2A		GOOD RESOLUTION
(LGG)	L	4	ST	2A		GOOD RESOLUTION
(LY)	L	4,8*	ST	3A		
PGD	L	6	ST	1A	P	GOOD RESOLUTION, STABLE MIGRATION
PGM	L	6	ST	2A		Pgm-2 VARIABLE

15 ENZYMES INVESTIGATES, REPRESENTING 24 PRESUMED NUMBER OF LOCI, 4 SUSPECTED POLYMORPHIC LOCI.

APPENDIX 4 : DETAILS OF BUFFERS, STAINS AND BIOCHEMICALS USED IN THEIR PREPARATION

TABLE 4.1 : Electrophoresis Buffer Recipes

TBE pH 9			
ELECTROSTARCH	Electrode Buffer	3.96 g	Boric acid
		1.64 g	Na_2EDTA
		38.75 g	Tris
		to 4 l	Milli Q water
	Gel Buffer		as Electrode Buffer
	Run Conditions		300V for 2h, 350V for next 3h
STARCH ESTERASE			
ELECTROSTARCH	Electrode Stock	12 g	LiOH
		118 g	Boric Acid
		to 1 l	Milli Q water
	Electrode Buffer	100 ml	of Electrode Stock Solution
		to 1 l	Milli Q water
	Gel Stock	86.6 g	Tris
		48.4 g	Citric Acid
		to 1 l	Milli Q water
	Gel Buffer	26 ml	of Gel Stock Solution
		15 ml	of Electrode Stock Solution
		to 1 l	Milli Q water
	Run Conditions		Regulate on 35 mA (Voltage increases during run from 80V to 210V),5.5h.

TABLE 4.1 (Cont.)

TRIS GLYCINE pH 8.5			
CELLULOSE ACETATE	Buffer	30.3 g	Tris
		14.4 g	Glycine
		to 1 l	Milli Q water
	Run Conditions	200V,0.5 to 1h (depending upon the enzyme under investigation)	
POULIK			
ELECTROSTARCH	Electrode Buffer	76.4 g	Boric acid
		9.6 g	NaOH
		to 4 l	Milli Q water
	Gel Buffer	37.2 g	Tris
		4.2 g	Citric acid
		to 4 l	Milli Q water
	Run Conditions	Regulate on 35mA (voltage increases during run from 80V to 210V), 5.5h.	
TEB pH 7.8/$MgCl_2$			
CELLULOSE ACETATE	Buffer	1.8 g	Tris
		1.9 g	Na_2EDTA
		0.2 g	Boric Acid
		2.0 g	$MgCl_2$
		to 1 l	Milli Q water
	Run Conditions	200V, 0.5 to 1h (depending upon the enzyme under investigation)	
TM pH 7.8			
ELECTROSTARCH	Electrode Buffer	24.2 g	Tris
		9.2 g	Maleic acid
		to 2 l	Milli Q water
	Gel Buffer	100 ml	of Electrode Buffer
		to 1 l	Milli Q water
	Run Conditions	50mA, 200V, 4h.	
CELLULOSE ACETATE	Buffer	500 ml	of Electrode Buffer
		to 1 l	Milli Q water
	Run Conditions	200V, 0.5 to 1h (depending upon the enzyme under investigation)	

TABLE 4.1 (Cont.)

TEM pH 7.4		
ELECTROSTARCH	Stock Solution	60.55 g Tris 58.05 g Maleic Acid 18.6 g Na_2EDTA 10.15 g $MgCl_2$ 26.0 g NaOH to 1 l Milli Q water
	Electrode Buffer	200 ml of Stock Solution to 1 l Milli Q water
	Gel Buffer	20 ml of Stock Solution to 1 l Milli Q water
	Run Conditions	60V,16h.
CAEA pH 7.2		
ELECTROSTARCH	Electrode Buffer	17.5 g Citric Acid 24 ml Aminopropyldiethanolamine to 2 l Milli Q water
	Gel Buffer	50 ml of Electrode Buffer to 500 ml Milli Q water
	Run Conditions	50 mA,160V,4h.
CELLULOSE ACETATE	Buffer	500 ml of Electrode Buffer to 1 l Milli Q water
	Run Conditions	200V, 0.5 to 1h (depending upon the enzyme under investigation)
CIT PO_4 pH 6.4		
ELECTROSTARCH	Electrode Buffer	28.4 g Na_2HPO_4 10.0 g Citric Acid to 1 l Milli Q water
	Gel Buffer	50 ml of Electrode Buffer to 1 l Milli Q water
	Run Conditions	50 mA,200V,5h.

TABLE 4.1 (Cont.)

CAM pH 6.1			
ELECTROSTARCH	Electrode Buffer	16.8 g	Citric acid
		19.5 ml	N-(3-aminopropyl)-morpholine
		to 2 l	Milli Q water
	Gel Buffer	25 ml	of Electrode Buffer
		to 500 ml	Milli-Q Water
	Run Conditions	50mA, 190V, 4h.	
CELLULOSE ACETATE	Buffer	500 ml	of electrode buffer
		to 1 l	Milli Q water
	Run Conditions	200V, 0.5 to 1h (depending upon the enzyme under investigation)	
TC pH 5.8			
ELECTROSTARCH	Electrode Buffer	131.2 g	Tris
		84.1 g	Citric Acid
		to 4 l	Milli Q water
	Gel Buffer	70 ml	Electrode Buffer
		to 2 l	Milli-Q Water
	Run Conditions	50mA,200V,4h.	

TABLE 4.2 : Sample Preparation Buffer Recipes

HOMOGENIZING BUFFER			
		0.1 ml	Mercaptoethanol
	to	100 ml	0.1 M Tris pH 8
EXTRACTION BUFFER			
		0.1 ml	Mercaptoethanol
		0.1 ml	Triton X-100
	to	100 ml	0.1 M Tris pH 8

TABLE 4.3 : Staining Buffer Recipes

0.2 M Na Citrate pH 4

8.4 g Citric Acid
to 180 ml Milli Q water
titrate to pH 4 with NaOH
to final volume of 200 ml Milli Q water

0.1 M Acetate pH 5

5.7 ml Glacial Acetic Acid
to 800 ml Milli Q water
titrate to pH 5 with NaOH
to final volume of 1 l Milli Q water

0.1 M Phosphate pH 6.7

13.6 g KH_2PO_4
to 800 ml Milli Q water
titrate to pH 6.7 with KOH
to final volume of 1 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 l Milli Q water

0.1 M Phosphate pH 7.5

200 ml 0.5 M NaH_2PO_4
to 800 ml Milli Q water
titrate to pH 7.5 with NaOH
to final volume of 1 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 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 l Milli Q water

TABLE 4.4 : Enzyme - Specific Histochemical Staining Recipes (modified from Harris and Hopkinson, 1978 and Shaw and Prasad,1970).

Note : Pyruvate and pyrazole is included in all formizan stain recipes containing NAD or NADP as we found that some NAD contamination occurs upon storage of stock solution of NADP.

ACONITASE (ACON)	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 (Results in pH 8.1)	80 ml

ACID PHOSPHATASE (ACPH)	EC 3.1.3.2

Note: For high pH gels, preincubate gel slice for 30 mins. in 0.5 M Boric Acid

alpha-Na-naphthyl acid phosphate	50 mg
0.1 M Acetate pH 5.0	20 ml
Fast Garnett GBC salt (purified grade)	10 mg
2% AGAR	20 ml

TABLE 4.4 (Cont.)

ACID PHOSPHATASE (ACPH) EC 3.1.3.2

(Alternative Recipe)

Note: For high pH gels, preincubate gel slice for 30 mins. in 0.5 M Boric Acid

4-methylumbelliferyl phosphate	20 mg
0.1 M Acetate pH 5.0	10 ml

Apply stain on filter paper overlay, incubate at 37° for 30 mins.(5-90 mins depending on activity). Remove filter paper. View under long UV. To stop the reaction & increase fluorescence, pipette a small amount of 1:4 ammonia onto gel.

ADENOSINE DEAMINASE (ADA) EC 3.5.4.4

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

TABLE 4.4 (Cont.)

ADENYLATE KINASE (AK)	EC 2.7.4.3
Glucose	100 mg
ADP	50 mg
0.2 M Tris-HCL pH 8	10 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	6 ml
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-Pruvate	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

ALKALINE PHOSPHATASE (ALKPH)	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

ASPARTATE AMINOTRANSFERASE (AAT)	EC 2.6.1.1
AAT Substrate Solution	20 ml
Add just before use:	
Fast Blue BB Salt (purified grade)	10 mg
2% AGAR	20 ml

TABLE 4.4 (Cont.)

Aspartate Aminotransferase Substrate Solution

alpha-Ketoglutaric Acid		0.292 g
L-Aspartic Acid		1.064 g
Polyvinylpyrrolidone		4.000 g
Na_2 EDTA		0.400 g
Na_2 H PO_4		11.360 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

CARBONIC ANHYDRASE (CA) EC 4.2.1.1

Stain: 1% bromothymol blue in pH 9-10 buffer.

Cover gel surface for 15 mins (or until gel becomes blue) with filter paper soaked in bromothymol blue. Remove paper and hose CO_2 onto the surface of the gel.
Yellow zones of carbonic anhydrase activity appear against a blue background.
To slow down enzyme activity, put gel over a block of ice.

CATALASE (CAT) EC 1.11.1.6

3% H_2O_2		10 ml
	OR	
1ml of conc soln		
Water	to	60 ml

Pour over gel and allow to stand for 30 sec.
Rinse gel under running water.
Pour acidified 1.5% KI solution over gel
Decant immediately white bands appear
Rinse gel under running water
Photograph immediately.

TABLE 4.4 (Cont.)

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	10 ml
NADP	1.5 ml
0.1M $MgCl_2$	0.5 ml
Hexokinase	160 u
G-6-P-DH	8 ml
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

D-ASPARTATE OXIDASE (DASOX)	EC 1.4.3.1
D-aspartic acid	200 mg
0.2 M Tris-HCL pH 8	10 ml
(Adjust to pH 8 with unbuffered 2M Tris)	
FAD	8 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	5 ml
NADH	30 mg
MTT	1 ml
2,6-dichlorophenol	0.75 ml
Water to	50 ml

TABLE 4.4 (Cont.)

ENOLASE (ENOL)	EC 4.2.1.11
2-Phosphoglyceric Acid	3 mg
ADP	5 mg
0.5 M TRIS-HCl pH 7	5 ml
1M $MgCl_2$	0.1 ml
NADH	5 mg
Lactate dehydrogenase	50 ul
Pyruvate kinase	30 ul

Apply on filter paper overlay.
View under UV.

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

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 (FDP)	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	5 ml
Glucose-6-Phosphate DH	3 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

TABLE 4.4 (Cont.)

FUMARASE (FUM) (Fumarate Hydratase)	EC 4.2.1.2
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

alpha-GALACTOSIDASE (alpha-GAL)	EC 3.2.1.22
4-Methylumbelliferyl-alpha-Galactoside	10 mg
0.2 M Na-Citrate pH 4.6	5 ml

Filter paper overlay
Visualise under U.V. light
Stop reaction with NH_4OH.

beta-GALACTOSIDASE (beta-GAL)	EC 3.2.1.23
4-Methylumbelliferyl-beta-Galactoside	5 mg
0.2 M Na-Citrate pH 4.6	10 ml

Filter paper overlay
Visualise under U.V. light
Stop reaction with NH_4OH.

TABLE 4.4 (Cont.)

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

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 (alpha-GLU)	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

TABLE 4.4 (Cont.)

GLUCOSEPHOSPHATE ISOMERASE (GPI)	EC 5.3.1.9
(Glucose-6-phosphate Isomerase)	
Fructose-6-phosphate	40 mg
0.2 M Tris-HCL pH 8	6 ml
NADP	0.2 ml
0.1 M $MgCl_2$	0.1 ml
Glucose-6-Phosphate DH	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

beta-GLUCURONIDASE (beta-GUS)	EC 3.2.1.31
4-Methylumbelliferyl-beta-D-Glucuronide	5 mg
0.2 M Na-Citrate pH 4.6	10 ml

Filter paper overlay
Visualise under U.V. light
Stop reaction with NH_4OH.

GLUTAMATE DEHYDROGENASE (GLUD)	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

GLUTAMATE PYRUVATE TRANSAMINASE (GPT)	EC 2.6.1.2
(Alanine Aminotransferase)	
DL alanine	50 mg
alpha-ketoglutaric acid	50 mg
0.2 M Tris-HCL pH 8 (Check pH of soln.)	10 ml
NADH	20 mg
LDH	100 u

Filter paper overlay
Visualise under U.V. light
Counter-stain with pH 8 Tris / MTT / PMS

TABLE 4.4 (Cont.)

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GA3PDH) EC 1.2.1.12

To prepare substrate:
Incubate at 37°C for 1 hour in....

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

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
Na_2EDTA	75 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

TABLE 4.4 (Cont.)

GLYCOLATE OXIDASE (GOX) ((S)-2-Hydroxy-acid Oxidase)	EC 1.1.3.15
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) (Alternative Recipe)	EC 1.1.3.1
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 I (GLO 1) (Lactoylglutathione lyase)	EC 4.4.1.5
Preincubate gel slice for 40 min in the following...	
Glutathione (reduced)	125 mg
0.1 M Phosphate pH 6.7	40 ml
Methylglyoxal	0.5 ml
MTT	2 ml
Then add...	
0.2 M Tris-HCL pH 8	10 ml
DCIP	5 mg

GLYOXALASE (Glo I) (Alternative recipe)	EC 4.4.1.5
Glutathione (reduced)	40 mg
0.1 M Phosphate pH 6.7	12 ml
Methylglyoxal	0.5 ml

Apply on filter paper overlay and incubate for 40 mins.

Remove filter, blot gel free of reaction mixture and add agar overlay.

Agar overlay: Iodine 1 gram
KI 3 g.
Water to 100 ml

Use 1.3 ml of this mixture to 30 ml 1 % agar at 45°C

TABLE 4.4 (Cont.)

GLYOXALASE II (GLO II) (Hydroxyacylglutathione Hydrolase)	EC 3.1.2.6
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	30 u
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

GUANYLATE KINASE (GUK)	EC 2.7.4.8
ATP	10 mg
GMP	25 mg
Phosphoenolpyruvate	10 mg
0.2 M Tris-HCl pH 8	6 ml
0.1 M $MgCl_2$	2 ml
0.5 M KCl	2 ml
0.5 M $CaCl_2$	0.2 ml
Pyruvate Kinase	10 u
Lactate Dehydrogenase	140 u
NADH	10 mg

Filter paper overlay
Visualise under U.V. light
Counter-stain with pH 8 Tris / MTT / PMS

TABLE 4.4 (Cont.)

HEXOKINASE (HK)	EC 2.7.1.1
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	2 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

HEXOSAMINIDASE (HEX) (B-N-Acetylglucosaminidase)	EC 3.2.1.52
NAG (Naphthol-AS-BI-2-acetamido--2-deoxy-B-D-glucopyranoside)	20 mg
Methanol (Absolute) (Gently Heat)	10 ml
0.1 M Acetate pH 5.0	20 ml
Fast Garnett GBC Salt (Purified Grade)	10 mg
2% AGAR	20 ml

HYDROXYBUTYRATE DEHYDROGENASE (HBDH)	EC 1.1.1.30
DL-beta-Hydroxybutyric Acid	630 mg
NaCl	287 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

(Note: gamma-Hydroxybutyric Acid EC 1.1.1.61)

TABLE 4.4 (Cont.)

ISOCITRATE DEHYDROGENASE (IDH)	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

LEUCINE AMINO PEPTIDASE (LAP)	EC 3.4.11.1
(Cytosol Aminopeptidase)	
Note: For high pH gels, preincubate gel slice for 30 mins in 0.5 M Boric Acid.	
L-leucyl-B-napthylamide	40 mg
0.1 M Acetate pH 5	20 ml
Fast Black K salt	20 mg

MALATE DEHYDROGENASE (MDH)	EC 1.1.1.37
0.5 M Tris-HCL pH 7	5 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

TABLE 4.4 (Cont.)

Na-L-Malate Substrate Solution

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

MALIC ENZYME (ME) EC 1.1.1.40

0.5 M Tris-HCL pH 7	5 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
NADP	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	5 ml
Mannose-6-phosphate	20 mg
NADP	1 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
Phosphoglucoseisomerase	8 ml
Glucose-6-Phosphate DH	6 ml
MTT	0.5 ml
PMS	0.2 ml
2 % AGAR	20 ml

TABLE 4.4 (Cont.)

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	2 ml
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.

PEPTIDASES

There are a no. of peptidases (see H & H) called A,B,C,D,E,F & S in mammals, apparently determined by separate loci that have characteristic but overlapping substrate specificities. Pep D is exceptional as it appears to be specific for dipeptides with proline (or hydroxyproline) as carboxy-terminal aa.

	S	A	B	C	D	E
LEU LEU LEU	+++	-	++	-	-	+
LEU VAL						
VAL LEU	++	+++	-	-	-	-
LEU TYR	+++	+++	++	+++	-	-
LEU GLY GLY	+	-	+++	-	-	-
LEU-PRO/PHE-PRO	-	-	-	-	++	-

TABLE 4.4 (Cont.)

PHOSPHOGLUCONATE DEHYDROGENASE (PGD)	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

PYRUVATE KINASE (PK)	EC 2.7.1.40
0.2 M Tris-HCL pH 8	5 ml
Phosphoenolpyruvate	8 mg
ADP	10 mg
Fructose-1,6-diPhosphate	15 mg
$MgSO_4$/KCL	0.5 ml
NADH	5 mg
LDH	50 u
Apply a filter paper overlay	
View under U.V. light	
Counter-stain with pH 8 Tris / MTT / PMS	

SORBITOL DEHYDROGENASE (SDH)	EC 1.1.1.14
(L-Iditol Dehydrogenase)	
0.2 M Tris-HCL pH 8	20 ml
D-Sorbitol	250 mg
NAD	2 ml
Na-Pyruvate	1 ml
Pyrazole	1 ml
MTT	0.5 ml
PMS	0.2 ml
2% AGAR	20 ml

TABLE 4.4 (Cont.)

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

TABLE 4.5 : Enzyme Stain Recipes For Cellulose Acetate

Note : Use filter paper overlay for all stains

ADENOSINE DEAMINASE (ADA)	EC 3.5.4.4
0.1 M Tris-HCl pH 8	1 ml
Adenosine	10 mg
Na Arsenate	0.5 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
Xanthine oxidase	0.1 u
Nucleoside phosphorylase	0.1 u
MTT	0.1 ml
PMS	0.1 ml

ALCOHOL DEHYDROGENASE (ADH)	EC 1.1.1.1
0.1 M Tris-HCl pH 8	1 ml
95% Ethanol	0.2 ml
NAD	0.2 ml
Na Pyruvate	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

ALDOLASE (ALD)	EC 4.1.2.13
0.1 M Tris-HCl pH 8	1 ml
Fructose-1,6-diphosphate	10 mg
Na Arsenate	4 ul
NAD	0.2 ml
Na Pyruvate	0.1 ml
Pyrazole	0.1 ml
Triosephosphate isomerase	5 u
Glyceraldehyde-3-phosphate DH	5 u
MTT	0.1 ml
PMS	0.1 ml

ASPARTATE AMINOTRANSFERASE (AAT)	EC 2.6.1.1
AAT Substrate Solution	1 ml
Water	1 ml
Fast Blue BB Salt	5 mg

TABLE 4.5 (Cont.)

ADENYLATE KINASE (AK)	EC 2.7.4.3
0.1 M Tris-HCl pH 8	1 ml
ADP	5 mg
Glucose	2 mg
$MgCl_2$	0.1 ml
NADP	0.2 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
Hexokinase	40 u
Glucose-6-phosphate DH	40 u
MTT	0.1 ml
PMS	0.1 ml

D-AMINO ACID OXIDASE (DAMOX)	EC 1.4.3.3
0.1 M Tris-HCl pH 8	2 ml
D-Leucine	20 mg
FAD	1 mg
Peroxidase	1 mg
3-amino-9-ethyl carbazole	0.1 ml

DIAPHORASE (DIA)	EC 1.6.2.2
0.1 M Tris-HCl pH 8	2 ml
NADH	2 mg
DCIP	0.1 ml
MTT	0.1 ml

(Clear background after bands appear with 1 M HCl)

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH)	EC 1.1.1.49
0.1 M Tris-HCl pH 8	1 ml
Glucose-6-phosphate	10 mg
$MgCl_2$	0.1 ml
NADP	0.2 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

TABLE 4.5 (Cont.)

GLYCOLATE OXIDASE (GOX)	EC 1.1.3.15
((S)-2-Hydroxy-acid Oxidase)	
0.1 M Tris-HCl pH 8	1 ml
Glycolic acid	5 mg
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

GLUCOSE-PHOSPHATE ISOMERASE (GPI)	EC 1.1.1.49
(Glucose-6-phosphate Isomerase)	
0.1 M Tris-HCl pH 8	1 ml
Fructose-6-phosphate	2 mg
$MgCl_2$	0.1 ml
NADP	0.2 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
Glucose-6-phosphate DH	4 u
MTT	0.1 ml
PMS	0.1 ml

HAEMOGLOBIN

Float gel-side-down in a 1% solution of amido black till protein bands disappear
Destain in several washes of fixative

HYDROXYACYL COENZYME A DEHYDROGENASE (HADH)	EC 1.1.1.35
0.1M Acetate pH 5	0.2 ml
Acetoacetyl CoA	0.6 ml
NADH	2 mg
Visualize under U.V. light	

ISOCITRATE DEHYDROGENASE (IDH)	EC 1.1.1.42
0.1 M Tris-HCl pH 8	1 ml
DL-Isocitrate	0.5 ml
NADP	0.2 ml
$MgCl_2$	0.1 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

TABLE 4.5 (Cont.)

LACTATE DEHYDROGENASE (LDH)	EC 1.1.1.27
0.1 M Tris-HCl pH 8	1 ml
70 % Na Lactate	0.2 ml
NAD	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

MALATE DEHYDROGENASE (MDH)	EC 1.1.1.37
0.1 M Tris-HCl pH 8	1 ml
Na Malate	0.2 ml
NAD	0.1 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

MALIC ENZYME (ME)	EC 1.1.1.40
0.1 M Tris-HCl pH 8	1 ml
Na-Malate	0.2 ml
NADP	0.2 ml
$MgCl_2$	0.1 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

MANNOSE-PHOSPHATE ISOMERASE (MPI)	EC 5.3.1.8
0.1 M Tris-HCl pH 8	1 ml
Mannose-6-phosphate	5 mg
NADP	0.2 ml
$MgCl_2$	0.1 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
Glucose-phosphate isomerase	8 u
Glucose-6-phosphate DH	6 u
MTT	0.1 ml
PMS	0.1 ml

TABLE 4.5 (Cont.)

PEPTIDASE (PEP)	EC 3.4.11
0.1 M Phosphate pH 7.5	1 ml
Dipeptide (FP or PL used)	5 mg
0.1 M $MgCl_2$	0.1 ml
Peroxidase	2 mg
Amino acid oxidase	1 mg
o-Dianisidine	0.1 ml

PHOSPHOGLUCONATE DEHYDROGENASE (PGD)	EC 1.1.1.44
0.1 M Tris-HCl pH 8	1 ml
6-Phosphogluconic acid	5 mg
NADP	0.2 ml
$MgCl_2$	0.1 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
MTT	0.1 ml
PMS	

PHOSPHOGLUCOMUTASE (PGM)	EC 5.4.2.2
0.1 M Tris-HCl pH 8	1 ml
Glucose-1-phosphate	15 mg
NADP	0.2 ml
$MgCl_2$	0.1 ml
Na pyruvate	0.1 ml
Pyrazole	0.1 ml
Glucose-6-phosphate DH	2 u
MTT	0.1 ml
PMS	0.1 ml

TABLE 4.6 : Stock Solutions Used In Enzyme-Specific Stain Recipes

SOLUTION	CONCENTRATION
Acetoacetyl CoA	2.5mg/ml
o-Dianisidine	10mg/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
Fixative	
Methanol : Acetic acid : Water	Ratio 4 : 1 : 5

TABLE 4.7 : Biochemicals and Other Products Used in This Investigation

BIOCHEMICALS:

cis-ACONITIC ACID	A-7251	(Sigma)
ADENOSINE	102075	(Boehringer)
ADENOSINE DEAMINASE	A-0387	(Sigma Type III)
ADENOSINE 5'-DIPHOSHATE	A-6521	(Sigma)
ADENOSINE 5'-TRIPHOSPHATE	A-5394	(Sigma)
	A-6144	(Sigma)
AGAROSE IEF	£-17-0468-01	(Pharmicia)
DL-ALANINE	A-7502	(Sigma)
L-ALANINE	A-7627	(Sigma)
L-ALANYL-L-PROLINE	A-3253	(Sigma)
ALDOLASE	A-6253	(Sigma Type I)
	£-102652	(Boehringer)
L-AMINO ACID OXIDASE	A-9253	(Sigma)
gamma-AMINO-n-BUTYRIC ACID	A-2129	(Sigma)
3-AMINO-9-ETHYL-CARBAZOLE	A-5754	(Sigma)
L-ARGININE	A-5006	(Sigma)
D-ASPARTIC ACID	A-8881	(Sigma)
5-BROMO-2'-DEOXYURIDINE	B-5002	(Sigma)
p-BROMOPHENOL	B-8502	(Sigma)
BROMOTHYMOL BLUE	B-0128	(Sigma)
CARBAMYL PHOSPHATE	C-5625	(Sigma)
CREATINE	C-3630	(Sigma)
CREATINE PHOSPHATE	C-6507	(Sigma)
L-CYSTEINE SULFINIC ACID	C-8380	(Sigma)
CYTIDINE	C-9505	(Sigma)
CYTIDINE 5'-TRIPHOSPHATE	C-1759	(Sigma)
P1,P5-DI(ADENOSINE-5'-)PENTAPHuSPHATE	D-4022	(Sigma)
3,3'-DIAMINOBENZIDINE	D-8126	(Sigma)
o-DIANISIDINE	D-3252	(Sigma)
2,6-DICHLOROPHENOL-IODOPHENOL	D-1878	(Sigma)
L-beta-3,4-DIHYDROXY-PHENYLALANINE	D-9628	(Sigma)
DL-DITHIOTHREITOL	D-0632	(Sigma)
FAST BLUE BB SALT Purified Grade :	F-3378	(Sigma)
FAST GARNET GBC SALT Purified Grade :	F-6504	(Sigma)
FLAVIN ADENINE DINUCLEOTIDE (FAD)	F-6625	(Sigma)
FLUORESCEIN DIACETATE	F-5502	(Sigma)
D-FRUCTOSE-1,6-DIPHOSPHATE		
NA2+ Salt:	£-750-1	(Sigma)
NH4+ Salt:	£-752-1	"
NH4+ Salt:	F-0752	"
D-FRUCTOSE-6-PHOSPHATE	F-3627	(Sigma)
FUMARIC ACID	F-5627	(Sigma)
D-GALACTOSE	G-0750	(Sigma)
D-GALACTOSE-6-PHOSPHATE	G-1625	(Sigma)
alpha-D-GLUCOSE-1-PHOSPHATE	G-1259	(Sigma GRADE VI)
	G-7000	(Sigma GRADE III)
D-GLUCONIC ACID	G-9005	(Sigma GRADE IX)
D-GLUCONIC ACID LACTONE	G-9005	(Sigma)
GLUCOSE OXIDASE	G-6500	(Sigma TYPE V)

TABLE 4.7 (Cont.)

D-GLUCOSE-6-PHOSPHATE	G-7879	(Sigma)
GLUCOSE-6-PHOSPHATE DEHYDROGENASE	G-8878	(Sigma)
	G-7878	(Sigma)
D-GLUTAMIC ACID	G-1001	(Sigma)
L-GLUTAMIC ACID	G-1626	(Sigma)
L-GLUTAMIC DEHYDROGENASE	G-2501	(Sigma TYPE I)
GLUTATHIONE OXIDIZED FORM:	G-4501	(Sigma)
REDUCED FORM:	G-4251	(Sigma)
GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE		
	G-5126	(Sigma)
	G-0763	"
	G-8380	"
DL-alpha-GLYCEROPHOSPHATE	G-6126	(Sigma)
alpha-GLYCEROPHOSPHATE DEHYDROGENASE	G-6751	(Sigma)
GLYOXALASE I	G-4252	(Sigma)
GLYCOLIC ACID	G-1884	(Sigma)
GLYCYL-L-LEUCINE	G-2002	(Sigma)
GUANINE	G-0381	(Sigma)
GUANOSINE 5'-MONOPHOSPHORIC ACID	G-8377	(Sigma)
HEXOKINASE	H-5625	(Sigma)
DL-alpha-HYDROXYBUTYRIC ACID	H-1253	(Sigma)
DL-beta-HYDROXYBUTYRIC ACID	H-6501	(Sigma)
DL-gamma-HYDROXYBUTYRIC ACID	H-3635	(Sigma)
DL-alpha-HYDROXY-ISOCAPROIC ACID	H-9251	(Sigma)
5-HYDROXYTRYPTAMINE	H-5755	(Sigma)
HYDROXYLAMINE	H-9876	(Sigma)
HYPOXANTHINE	H-9377	(Sigma)
INOSINE	I-4125	(Sigma)
ISOCITRATE DEHYDROGENASE	I-2002	(Sigma)
DL-ISOCITRIC ACID	I-1252	(Sigma)
alpha-KETOGLUTARIC ACID	K-1750	(Sigma)
alpha-KETOVALERIC ACID	K-2625	(Sigma)
LACTATE DEHYDROGENASE	L-1254	(Sigma)
	£-127230	(Boehringer)
L(+)LACTIC ACID	L-2000	(Sigma)
D-LEUCINE	L-7750	(Sigma)
L-LEUCINE-beta-NAPHTHYLAMIDE HCL	L-0376	(Sigma)
L-LEUCYL-L-ALANINE	L-9250	(Sigma)
L-LEUCYLGLYCYL-GLYCINE	L-9750	(Sigma)
L-LEUCYL-L-LEUCINE	L-2752	(Sigma)
L-LEUCYL-L-LEUCYL-L-LEUCINE	L-0879	(Sigma)
L-LEUCYL-L-TYROSINE	L-0501	(Sigma)
L-LEUCYL-L-VALINE	L-1377	(Sigma)
L-LYSINE	L-5501	(Sigma)
L-LYSYL-L-LEUCINE	L-1879	(Sigma)
MALIC DEHYDROGENASE	M-9004	(Sigma)
D-MANNOSE-6-PHOSPHATE		
Disodium Salt:	M-6876	(Sigma)
Barium Salt :	M-8754	(Sigma)
D-METHIONINE	M-9375	(Sigma)
4-METHYLUMBELLIFERYL ACETATE	M-0883	(Sigma)
4-METHYLUMBELLIFERYL-alpha-D-GALACTOSIDE		
	M-7633	(Sigma)
4-METHYLUMBELLIFERYL-beta-D-GALACTOSIDE	M-1633	(Sigma)

TABLE 4.7 (Cont.)

4-METHYLUMBELLIFERYL-beta-D-GLUCURONIDE	M-9130	(Sigma)
4-METHYLUMBELLIFERYL-beta-D-GLUCOSIDE	M-9766	(Sigma)
4-MEHTYLUMBELLIFERYL-N-ACETYL-beta-D-GALACTOSAMINIDE	M-9129	(Sigma)
4-METHYLUMBELLIFERYL PHOSPHATE	M-8883	(Sigma)
4-METHYLUMBELLIFERYL SULFATE	M-7133	(Sigma)
MTT (TETRAZOLIUM SALT)	M-2128	(Sigma)
alpha-NAPHTHYL ACETATE	N-6750	(Sigma)
beta-NAPHTHYL ACETATE	N-6875	(Sigma)
alpha-NAPHTHYL ACID PHOSPHATE	N-7000	(Sigma)
NAPHTHYL-AS-BI-ACETYL-beta-D-GLUCOSAMINIDE	N-4006	(Sigma)
alpha-NAPHTHYL BUTYRATE	N-8000	(Sigma)
alpha-NAPHTHYL PHOSPHATE	N-7255	(Sigma)
beta-NAPHTHYL PHOSPHATE	N-1132	(Sigma)
NITRO BLUE TETRAZOLIUM	N-6876	(Sigma GRADE III)
beta-NICOTINAMIDE ADENINE DINUCLEOTIDE	N-7381	(Sigma)
	N-7004	(Sigma)
beta-NICOTINAMIDE ADENINE DINUCLEOTIDE Reduced Form:	N-8129	(Sigma)
beta-NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE	N-0505	(Sigma)
NUCLEOSIDE PHOSPHATE	£-107956	(Boehringer)
L-ORNITHINE-HCl	O-2375	(Sigma)
OXALACETIC ACID	O-4126	(Sigma)
PEROXIDASE	P-8000	(Sigma)
PHARMALYTE (pH3-10)	17-0456-01	(Pharmacia)
PHENAZINE METHOSULFATE	P-9625	(Sigma)
PHENOLPHTHALEIN DIPHOSPHATE	P-9875	(Sigma)
D-PHENYLALANINE	P-1751	(Sigma)
L-PHENYLALANYL-L-LEUCINE	P-3876	(Sigma)
L-PHENYLALANYL-L-PROLINE	P-6258	(Sigma)
L-PHENYLALANYL-L-TYROSINE	P-4876	(Sigma)
L-PHENYLALANYL-L-VALINE	P-5001	(Sigma)
p-PHENYLENEDIAMINE	P-6001	(Sigma GRADE II)
PHOSPHOCREATINE	P-6502	(Sigma)
PHOSPHO (ENOL) PYRUVATE	P-7252	(Sigma)
6-PHOSPHOGLUCONIC ACID	P-6888	(Sigma GRADE III)
	P-7877	(Sigma GRADE IV)
PHOSPHOGLUCOSE ISOMERASE	P-5381	(Sigma TYPE III)
	P-9010	(Sigma TYPE X)
D(+)2-PHOSPHOGLYCERIC ACID	P-0257	(Sigma)
D(-)3-PHOSPHOGLYCERIC ACID	P-8627	(Sigma)
PIPES (pH range 6.1 to 7.5)	P-6757	(Sigma)
L-PROLINE	P-0380	(Sigma)
L-PROLYL-L-LEUCINE	P-1130	(Sigma)
L-PROLYL-L-PHENYLALANINE	P-1505	(Sigma)
PYRAZOLE	P-2646	(Sigma)
PYRIDOXAL-5'-PHOSPHATE	P-9255	(Sigma)
L-PYROGLUTAMIC ACID	P-3634	(Sigma)
PYRUVATE KINASE	P-1381	(Sigma TYPE I)
	P-9136	(Sigma TYPE III)
	£-128155	(Boehringer)
PYRUVIC ACID	P-2256	(Sigma)

TABLE 4.7 (Cont.)

SUCCINYLCHOLINE CHLORIDE	S-8251	(Sigma)
TAURINE	T-0625	(Sigma)
THIAMINE HCl	T-4625	(Sigma)
D(+)TREHALOSE DIHYDRATE	T-5251	(Sigma)
TRIOSEPHOSPHATE ISOMERASE	T-2391	(Sigma TYPE III)
	T-2507	(Sigma TYPE I)
TRIS:SIGMA 7-9 BIOCHEMICAL BUFFER	T-1378	(Sigma)
L-VALYL-L-ALANINE	V-1250	(Sigma)
L-VALYL-L-LEUCINE	V-1625	(Sigma)
XANTHINE OXIDASE	X-1875	(Sigma GRADE I)
	X-4875	(Sigma GRADE IV)
COMPUTING:		
APPLE PC	2+ , 2e	
MAINFRAMES	CYBER 170	
	VAX 11/785	
PLOTTER	MP1000	(Graphtec)
PRINTER	EPSON FX80+	
DISPOSABLES		
1.8ml NUNC CRYOTUBES	3-63401	(Medos)
1.5ml MICROCENTRIFUGE TUBES	96.2494.4.001	(Medos)
ELECTROPHORESIS SUPPORT MEDIA:		
AMPHOLINE PAG PLATES	1804-101	(LKB)
CELLOGEL	CHE-038	(Edwards)
ELECTROSTARCH	Lot No.392	(Electrostarch Co. Madison,Wisconsin ...no longer available)
TITAN III PLATES	3024	(Helena)
REFRIGERATION		
CRYOGENIC REFRIGERATORS	35VHC	(Taylor-Wharton)
PHOTOGRAPHY:		
DEVELOPER	D19	(Kodak)
PHOTOS		(ColorPro)
TECHNICAL PAN FILM	2415	(Kodak)
COLOUR SLIDE FILM	640-T	(3M)
POWER SUPPLIES:		
HEATHKIT	SP-17A	(Schlumberger)
PHARMACIA	EPS 500/400	(Pharmacia)
	EPS3000/150	"
VOLTHOUR INTEGRATOR	VH-1	"

APPENDIX 5: NOTES ON PROGRAMS USED IN STATISTICAL ANALYSIS OF ELECTROPHORETIC DATA.

Felsenstein's PHYLIP, Phylogeny Inference Package (Version 2.8)
(from PHYLIP Documentation).

Three types of programs were used in our analyses, one for gene frequency data calculated from starch gel electrophoresis of polymorphic loci (CONTML), one for distance matrices calculated from the gene frequency data (FITCH), and one for discrete characters scored from isoelectric focusing gels (MIX).

CONTML (continuous character maximum likelihood program) uses gene frequencies to construct estimates of the maximum likelihood evolutionary tree under the following assumptions:

1. Different lineages evolve independently;
2. After two lineages split, their genetic drift proceeds independently;
3. Each gene frequency changes by genetic drift;
4. Different loci drift independently.

Input Format:

(5 spaces) No. of Populations (5 spaces) No. of Loci

No. of Alleles at each Locus (in order with a space between each)

Population Name (9 characters or less) Allele frequencies minus one (in order with a space between each datum).

The program treats the input as gene frequencies at a series of loci, and square root transforms the allele frequencies, constructing the frequency of the missing allele at each locus first.

Output Format:

The topology of the tree is given by an unrooted tree diagram. The lengths (in expected amounts of variance) are given in a table below the topology, and a rough confidence interval given for each length. Negative lower bounds on length indicate that rearrangements may be acceptable at this point in the tree, (indicated by a dotted line in dendrograms plotted using CONPLOT). The units of length are amounts of expected accumulated variance. The log likelihood (natural log) of each tree is given, as is the number of topologies tried. The log likelihood allows a likelihood ratio hypothesis test (Sokal and Rohlf, 1981 pp.695-696).

FITCH (Fitch-Margoliash and Least-Squares Distance Methods) deals with data which comes in the form of pairwise distances between all pairs of taxa.

In analysing these data, the program implicitly assumes:

1. Each distance is measured independently from the others: no item of data contributes to more than one distance;
2. The distance between each pair of taxa is drawn from a distribution with an expectation which is the sum of values (in effect, amounts of evolution).

These two assumptions are dubious in the case of genetic distance from gene frequency data since additivity or independence will not be expected to be true. Therefore, CONTML is more appropriate. However, if genetic drift is the mechanism of divergence, additivity holds and FITCH will not give positively misleading results (i.e. will not make a statistically inconsistent estimate).

The branch lengths of the tree are unconstrained (by time).

Input Format:
(5 spaces)No. of Populations
Population Name (9 characters or less) followed by the set of distances to all other populations.

Output Format:
The output consists of an unrooted tree and the lengths of the interior segments. The sum of squares and average percent standard deviation is given, as well as the number of trees examined.

MIX (mixed method parsimony)
carries out the Wagner and Camin-Sokal parsimony methods, as specified for each discrete character. The program defaults to carrying out Wagner parsimony.

The two methods assume:
1. Ancestral states are known (Camin-Sokal) or unknown (Wagner).
2. Different characters evolve independently.
3. Different lineages evolve independently.
4. Changes 0 to 1 are more probable than 1 to 0 (Camin-Sokal) or equally probable (Wagner).
5. Both of these kinds of changes are "a priori" improbable over the evolutionary time spans involved in the differentiation of the group in question.
6. Other kinds of evolutionary event, such as retention of polymorphism, are far less probable than 0 to 1 changes.
7. Rates of evolution in different lineages are sufficiently low that two changes in a long segment of the tree are less probable than one change in a short segment.

Input Format:
(5spaces)No. of Species(5 spaces)No. of Characters
Species Name followed by the set of character states without a space between each.

Allowable characters states are "0", "1", "P", "B" and "?".

The data are coded into a series of two state characters ("0" or "1"), polymorphisms are indicated by "P"; if both characters are present this is indicated by "B"; missing data is indicated by "?", when the state is unknown or does not apply.

Output Format:
The tree is printed out as either rooted or unrooted, depending upon which is appropriate, followed by a table of the number of changes of state required for each character. With the Wagner option, it may not be possible to unambiguously locate places on the tree where changes occur, as there may be multiple possibilities. A table is printed out after the last tree, showing for each branch whether there are known to be changes in the branch.

APPENDIX 6 : DESCRIPTION OF ENZYME BANDING PATTERNS FOR THE POLYMORPHIC LOCI USED IN DISCRIMINATION OF STOCKS OF WHITING SPECIES

ALCOHOL DEHYDROGENASE (ADH) EC 1.1.1.1

ADH was examined in extracts of liver tissue, and migrates cathodally in CAM pH 6.1 buffer.

Subunit Structure : dimer.

Banding Pattern : single band in monomorphic fish (with a single cathodal sub-band sometimes present); heterozygotes for the scored "b" allele did not always show the expected 1:2:1 activity ratios expected for a dimeric protein.

Variation was detected for:
S. bassensis flindersi - 5 alleles, c (common), b (may represent more than one allele, clumped for statistical purposes), a, d & e (rare).

Figure 6.1 shows the observed banding patterns for *S. bassensis flindersi*.

ASPARTATE AMINOTRANSFERASE (AAT) EC 2.6.1.1

Aat-2 was examined in extracts of liver tissue, and migrates anodally in CAM pH6.1 buffer.

Subunit Structure : dimer

Banding Pattern : single band in monomorphic fish (with one or two anodal sub-bands); heterozygous individuals may or may not resolve into 3 clear bands (the expected pattern for a dimeric protein).

Low frequency variation was detected for:
S. bassensis flindersi - 3 alleles, b (common), a & c (rare).
S. bassensis bassensis - 2 alleles, b (common), a (rare).

Figure 6.2 shows the observed banding patterns for *S. bassensis bassensis* and for *S. bassensis flindersi*.

GLUCOSE-PHOSPHATE ISOMERASE (GPI) EC 5.3.1.9

A multilocus system, GPI has been reported as 2 loci in most fish with an hybrid heteropolymer zone of activity (Avise, 1973). However, the banding pattern observed in whiting species does not necessarily fit this hypothesis (see note below). For this reason, GPI has been interpreted here as representing 3 loci.

GPI was examined in extracts of muscle tissue, and all loci migrate anodally in Poulik buffer.

Subunit Structure : dimer (monomeric pattern for Gpi-2).

Banding Pattern : Gpi-1 is the major muscle component of this enzyme. Monomorphic fish show a single band with 2 anodal sub-bands appearing soon after; heterozygotes showed 3 bands (with one or two anodal sub-bands).

Low frequency variation was detected for:

S. bassensis bassensis - 3 alleles, b (common), a & c (rare);
S. bassensis flindersi - 3 alleles, b (common), a & c (rare);
S. punctata - 2 alleles, b (common), a (rare);
S. robusta - 2 alleles, a (common), b (rare);

Gpi-2 shows a single band in monomorphic fish; "heterozygotes" showed 2 bands.

Low frequency variation was detected for:

S. bassensis bassensis - 3 alleles b (common), a & c (rare);
S. bassensis flindersi - 6 alleles, c (common), a,b,d,e & f (rare);
S. punctata - 2 alleles, b (common), a (rare);
S. robusta - 3 alleles, b (common), a & c (rare);

Gpi-3 shows a single band in monomorphic fish (with one or two anodal sub-bands present in some samples); heterozygous individuals with alleles 3 mm apart do not resolve into visible bands; other heterozygotes showed 3 bands.

Low frequency variation detected for:

S. bassensis bassensis - 3 alleles, b (common), a & c (rare);
S. bassensis flindersi - 6 alleles, c (common), a,b,d,e & f (rare);
S. punctata - 3 alleles, c (common), a & b (rare);
S. robusta - 3 alleles, b (common), a & c (rare);

Figures 6.3 to 6.6 shows observed banding patterns for *S. bassensis bassensis*, *S. bassensis flindersi*, *S. punctata* and *S. robusta*.

Note The banding pattern expected to fit the hypothesis that Gpi-2 is actually an hybrid heteropolymer would be a single band when Gpi-1 and Gpi-3 are homozygous, 2 bands when either Gpi-1 or Gpi-3 are heterozygous, and 3 bands when both Gpi-1 and Gpi-3 are heterozygous.

However, when an heterozygous pattern appears at one or other of the fast or slow locus, one or two bands may be seen at the middle zone of activity. Conversely, when homozygous patterns appear at both the fast and slow loci, again one or two bands may be seen at the middle zone of activity. A 3-banded pattern at Gpi-2 was not observed.

GLUTAMATE-PYRUVATE TRANSAMINASE (GPT) EC 2.6.1.2

GPT was examined in extracts of liver tissue, and migrates anodally in Poulik buffer.
Subunit Structure: monomer.
Banding Pattern : single band in monomorphic fish; heterzygotes showed 2 bands.
Variation was detected for:
S. punctata - 3 alleles b or c (common), a (rare).

Figure 6.7 shows the observed banding patterns for *S. punctata*.

ISOCITRATE DEHYDROGENASE (IDH) EC 1.1.1.42

Idh-1 was examined in extracts of muscle tissue, and migrates anodally in CAM pH6.1 buffer.
Subunit Structure : dimer
Banding Pattern : single band in monomorphic fish; heterozygotes show 3 bands typical of a dimeric protein.
Variation was detected for:
S. robusta - 2 alleles, a & b.

Figure 6.8 shows the observed banding pattern for *S. robusta*.

MANNOSE-PHOSPHATE ISOMERASE (MPI) EC 5.3.1.8

MPI was examined in extracts of muscle tissue and migrates anodally in both CAM pH6.1 and Poulik buffers.
Subunit Structure : monomer
Banding Pattern : single band in monomorphic fish (with a single sub-band present in CAM pH6.1); heterozygotes show two bands.
Variation was detected for:
S. bassensis flindersi - 5 alleles b & c (common), a, d, e (rare)
S. robusta - 5 alleles d (common), a, b, c, and e (rare)

Figure 6.9 shows observed banding patterns of *S. bassensis flindersi*, and *S. robusta*.

PEPTIDASE-PL (PEPC) EC 3.4.11

PepC was examined in extracts of liver tissue and migrates anodally in CAM pH6.1 buffer.
Subunit Structure : monomer
Banding Pattern : single band in monomorphic fish; heterozygotes may or may not resolve into 2 clear bands (the expected pattern for a monomeric protein).
Low frequency variation was detected for:
S. bassensis bassensis - 3 alleles b (common) a & c (rare)
and *S. bassensis flindersi*, but due to the very low frequency of heterozygotes, was not used for statistical analysis.

Figure 6.10 shows observed banding pattern for *S. bassensis bassensis*.

PHOSPHOGLUCOMUTASE (PGM) EC 5.4.2.2

PGM was examined in extracts of muscle tissue and migrates anodally in Poulik buffer.
Subunit Structure : monomer
Banding Pattern : single band in monomorphic fish, heterozygotes show 2 bands typical of that expected for a monomeric protein.
Variation was detected for:
S. robusta Pgm-1 - 5 alleles b & c (common), a, d & e.
S. punctata Pgm-1 - 3 alleles b (common), a & c.
Pgm-2 - 4 alleles b (common), a, c & d.

Figures 6.11 & 6.12 show the observed banding patterns for *S. robusta* and *S. punctata*.

6-PHOSPHOGLUCONATE DEHYDROGENASE (PGD) EC 1.1.1.44

PGD was examined in extracts of liver tissue and migrates anodally in CAM pH6.1 buffer. It was found that use of the newly-aquired Pharmacia constant power supply (under conditions of constant current) stabilized (to a large extent) the warping of this enzyme's migration.
Subunit Structure : dimer
Banding Pattern : single band in monomorphic fish; heterozygotes (occasionally atypical) were of a 3-banded pattern.
Variation was detected for:
S. bassensis flindersi - 4 alleles, a & b (common), c & d (rare).
S. punctata - 2 alleles, a (common) & b.

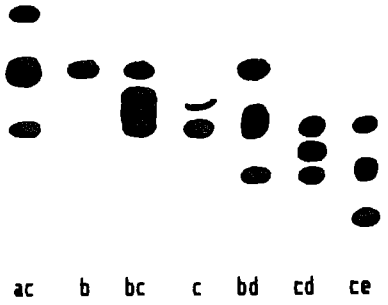
Figure 6.13 shows observed banding patterns for *S. bassensis flindersi* and *S. punctata*.

SORBITOL DEHYDROGENASE (SDH) EC 1.1.1.14

SDH was examined in extracts of liver tissue and migrates anodally in Poulik buffer.
Subunit Structure : Tetramer
Banding Pattern : single band in monomorphic fish (which occasionally sub-bands anodally); heterozyous individuals may or may not resolve into 5 clear bands (as expected for a tetrameric protein).
Variation was detected for:
S. robusta - 3 alleles a and b (common), & c.

Figure 6.14 shows observed banding patterns for *S. robusta*.

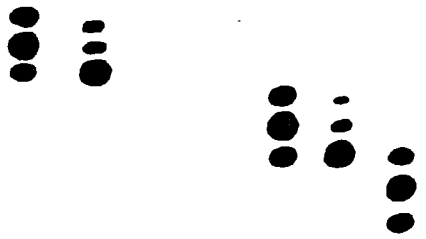
-ve



ac b bc c bd cd ce

Figure 6.1 : Observed banding patterns and genotypes designated for Adh-1 (EC 1.1.1.1) from *S. bassensis flindersi*

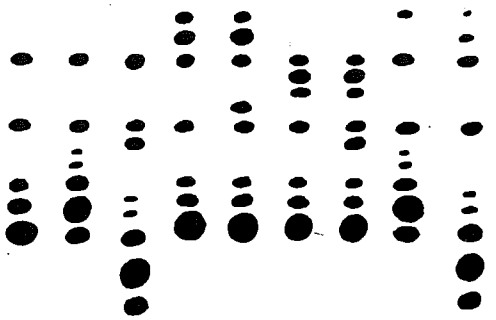
+ve



ab b ab b bc

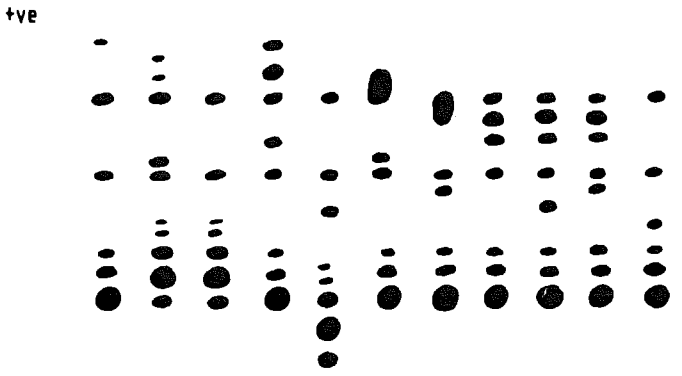
Figure 6.2 : Observed banding patterns and genotypes designated for Aat-2 (EC 2.6.1.1) from *S. bassensis bassensis* and *S. bassensis flindersi*, respectively.

+ve



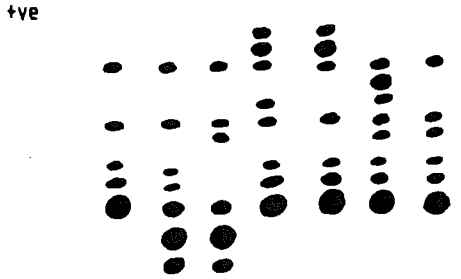
Gpi-3	b	b	b	ab	ab	bc	bc	b	b
Gpi-2	b	ab	bc	b	ab	b	bc	b	b
Gpi-1	b	ab	bc	b	b	b	b	ab	bc

Figure 6.3 : Observed banding patterns and genotypes designated for GPI (EC 5.3.1.9) from *S. bassensis bassensis*.



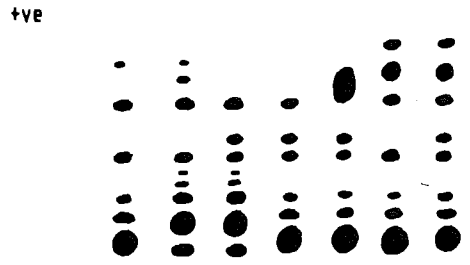
Gpi-3	c	c	c	ac	c	bc	cd	ce	ce	ce	c
Gpi-2	c	bc	c	ac	ce	bc	cd	c	ce	cd	cf
Gpi-1	b	ab	ab	b	bc	b	b	b	b	b	b

Figure 6.4 : Observed banding patterns and genotypes designated for GPI (EC 5.3.1.9) from *S. bassensis flindersi*.



Gpi-3	b	b	b	ab	ab	bc	b
Gpi-2	b	b	bc	ab	b	bc	bc
Gpi-1	a	ab	ab	a	a	a	a

Figure 6.5 : Observed banding patterns and genotypes designated for GPI (EC 5.3.1.9) from *S. robusta*.



Gpi-3	c	c	c	c	bc	ac	ac
Gpi-2	b	b	ab	ab	ab	b	ab
Gpi-1	b	ab	ab	b	b	b	b

Figure 6.6 : Observed banding patterns and genotypes designated for GPI (EC 5.3.1.9) from *S. punctata*.

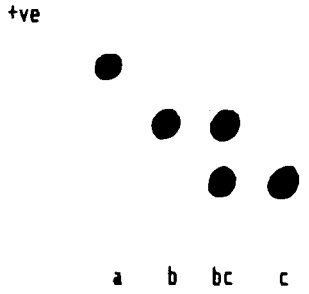


Figure 6.7 : Observed banding patterns and genotypes designated for GPT (EC 2.6.1.2) from *S. punctata*.

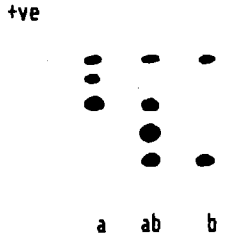


Figure 6.8 : Observed banding patterns and genotypes designated for IDH (EC 1.1.1.42) from *S. robusta*.

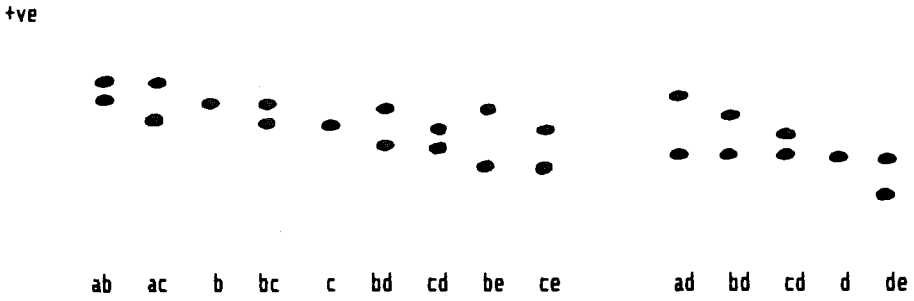


Figure 6.9 : Observed banding patterns and genotypes designated for MPI (EC 5.3.1.8) from *S. bassensis flindersi* and *S. robusta*, respectively.

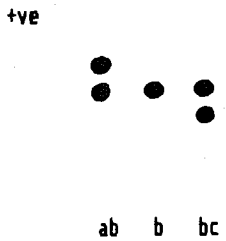


Figure 6.10 : Observed banding patterns and genotypes designated for Pep C (PL) (EC 3.4.11) from *S. bassensis bassensis*.

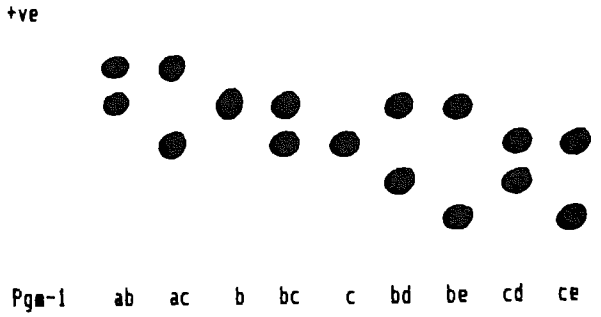


Figure 6.11 : Observed banding patterns and genotypes designated for PGM (EC 5.4.2.2) from *S. robusta*

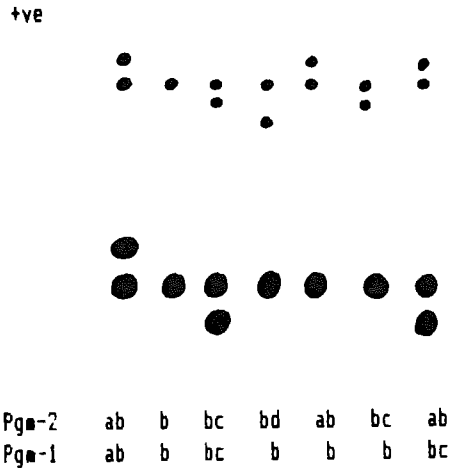


Figure 6.12 : Observed banding patterns and genotypes designated for PGM (EC 5.4.2.2) from *S. punctata*.

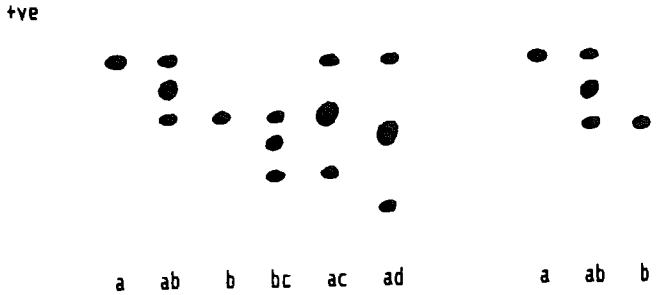


Figure 6.13 : Observed banding patterns and genotypes designated for PGD (EC 1.1.1.44) from *S. bassensis flindersi* and *S. punctata*, respectively.

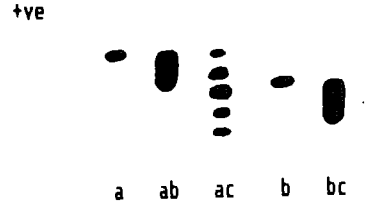


Figure 6.14 : Observed banding patterns and genotypes designated for SDH (EC 1.1.1.14) from *S. robusta*.

APPENDIX 7: GENE FREQUENCY INPUT DATA FILES FOR *S. bassensis bassensis*, *S. bassensis flindersi*, *S. punctata* and *S. robusta*, and G TESTS FOR *S. bassensis flindersi*.

NOTE For gene frequency input data, the numbers of alleles at each locus are indicated by the second line of figures ; the gene frequencies are entered in the order listed for each table ; one less figure is entered than the number of alleles at each locus because the programs used compute the last figure.

TABLE 7.1 : Gene Frequency Input Data For The Loci Pgd, Adh, Aat-2, Gpi-1, Gpi-2, Gpi-3 and Mpi of *S. bassensis flindersi* From Thirteen Localities in N.S.W., Six Localities in VIC., One Locality in TAS. and One Locality in S.A.

---

30 7 b
4 5 3 3 6 6 5
byronbay 0.743 0.257 0.000 0.000 0.135 0.838 0.014 0.000 1.000 0.000 1.000 0.0125 0.0125
0.975 0.000 0.000 0.000 0.000 1.000 0.000 0.000 0.000 0.7125 0.2875 0.000
evanshead 0.695 0.305 0.000 0.000 0.102 0.841 0.057 0.000 1.000 0.000 1.000 0.000 0.000
0.977 0.011 0.000 0.034 0.000 0.943 0.011 0.011 0.000 0.700 0.300 0.000
yamba1 0.601 0.393 0.005 0.000 0.178 0.792 0.030 0.011 0.986 0.003 0.994 0.000 0.003
0.985 0.006 0.006 0.000 0.003 0.991 0.000 0.006 0.000 0.670 0.327 0.003
yambalge 0.523 0.477 0.000 0.000 0.161 0.821 0.018 0.007 0.993 0.000 1.000 0.000 0.000
0.993 0.007 0.000 0.000 0.000 0.993 0.000 0.000 0.000 0.591 0.409 0.000
yambasml 0.669 0.325 0.006 0.000 0.163 0.798 0.039 0.015 0.979 0.000 0.990 0.000 0.005
0.985 0.005 0.005 0.000 0.005 0.990 0.000 0.005 0.000 0.781 0.213 0.006
yamba2 0.597 0.403 0.000 0.000 0.093 0.907 0.000 0.000 1.000 0.016 0.984 0.000 0.016
0.968 0.016 0.000 0.000 0.000 0.984 0.000 0.016 0.000 0.703 0.297 0.000
yamba3 0.686 0.314 0.000 0.000 0.143 0.816 0.041 0.000 0.991 0.000 1.000 0.000 0.009
0.991 0.000 0.000 0.009 0.000 0.991 0.000 0.000 0.009 0.717 0.274 0.000
wooli 0.702 0.298 0.000 0.000 0.133 0.828 0.039 0.006 0.994 0.000 1.000 0.005 0.000
0.962 0.033 0.000 0.006 0.017 0.960 0.017 0.000 0.005 0.703 0.292 0.000
nsolitary 0.600 0.394 0.006 0.000 0.138 0.846 0.016 0.000 1.000 0.000 0.995 0.010 0.030
0.940 0.020 0.000 0.040 0.010 0.935 0.010 0.005 0.005 0.687 0.303 0.000
coffsh1 0.698 0.302 0.000 0.000 0.156 0.844 0.000 0.010 0.990 0.000 1.000 0.000 0.000
1.000 0.000 0.000 0.000 0.000 1.000 0.000 0.000 0.000 0.611 0.367 0.022
coffsh2 0.558 0.419 0.023 0.012 0.171 0.805 0.012 0.000 1.000 0.000 1.000 0.000 0.012
0.988 0.000 0.000 0.000 0.000 1.000 0.000 0.000 0.000 0.295 0.705 0.000
camden1 0.599 0.396 0.005 0.000 0.172 0.796 0.032 0.000 1.000 0.000 1.000 0.000 0.005
0.965 0.020 0.005 0.000 0.005 0.965 0.005 0.020 0.017 0.600 0.383 0.000
camden2 0.469 0.531 0.000 0.000 0.046 0.880 0.068 0.000 0.988 0.000 1.000 0.013 0.000
0.974 0.013 0.000 0.000 0.013 0.974 0.013 0.000 0.040 0.697 0.250 0.013
forster1 0.564 0.436 0.000 0.000 0.059 0.898 0.042 0.005 0.995 0.000 1.000 0.000 0.000
0.985 0.010 0.005 0.000 0.000 0.985 0.010 0.005 0.005 0.538 0.429 0.027
forster2 0.647 0.353 0.000 0.000 0.1176 0.8676 0.0147 0.000 1.000 0.000 0.9875 0.000
0.000 0.949 0.032 0.019 0.000 0.000 0.9625 0.025 0.0125 0.0065 0.750 0.237 0.000
forster3 0.571 0.429 0.000 0.000 0.155 0.845 0.000 0.017 0.983 0.000 0.984 0.000 0.000
0.953 0.047 0.000 0.000 0.000 0.969 0.013 0.000 0.000 0.672 0.328 0.000

TABLE 7.1 (Cont.)

ptstephen 0.667 0.326 0.007 0.000 0.114 0.826 0.060 0.005 0.990 0.000 1.000 0.000 0.010
0.980 0.010 0.000 0.000 0.010 0.980 0.010 0.000 0.000 0.561 0.439 0.000
newcastle 0.750 0.241 0.007 0.000 0.114 0.826 0.060 0.005 0.990 0.000 1.000 0.000 0.010
0.980 0.010 0.000 0.000 0.010 0.980 0.010 0.000 0.000 0.561 0.439 0.000
sydney 0.611 0.383 0.006 0.000 0.190 0.792 0.018 0.017 0.978 0.000 1.000 0.000 0.006
0.974 0.019 0.001 0.013 0.000 0.974 0.013 0.000 0.000 0.635 0.365 0.000
jervisbay 0.617 0.383 0.000 0.000 0.106 0.874 0.020 0.010 0.990 0.000 1.000 0.005 0.010
0.980 0.000 0.005 0.0101 0.0101 0.9747 0.000 0.0051 0.005 0.630 0.360 0.005
eden 0.612 0.388 0.000 0.000 0.218 0.739 0.043 0.021 0.969 0.000 1.000 0.000 0.000
0.989 0.011 0.000 0.000 0.000 0.9894 0.0053 0.0053 0.000 0.671 0.324 0.005
lakesent 0.651 0.349 0.000 0.000 0.162 0.823 0.015 0.000 1.000 0.000 1.000 0.000 0.000
0.974 0.011 0.015 0.0163 0.000 0.9565 0.0109 0.0163 0.000 0.636 0.353 0.005
sanremo 0.589 0.411 0.000 0.000 0.136 0.847 0.017 0.000 1.000 0.000 1.000 0.000 0.000
0.974 0.011 0.015 0.0163 0.000 0.9565 0.0109 0.0163 0.000 0.636 0.353 0.005
ptlonsdal 0.657 0.343 0.000 0.000 0.079 0.895 0.026 0.000 1.000 0.000 1.000 0.000 0.000
0.952 0.024 0.024 0.0179 0.000 0.9196 0.0357 0.0268 0.000 0.690 0.298 0.012
cpatton1 0.653 0.341 0.006 0.000 0.126 0.846 0.028 0.016 0.973 0.000 1.000 0.000 0.006
0.994 0.000 0.000 0.033 0.022 0.945 0.000 0.000 0.000 0.652 0.347 0.000
cpatton2 0.647 0.332 0.016 0.000 0.170 0.797 0.027 0.000 1.000 0.000 1.000 0.000 0.000
0.985 0.015 0.000 0.000 0.000 0.983 0.006 0.011 0.000 0.640 0.349 0.005
apollobay 0.582 0.405 0.006 0.000 0.114 0.848 0.038 0.000 1.000 0.006 0.994 0.000 0.006
0.949 0.026 0.019 0.000 0.000 0.942 0.026 0.019 0.000 0.627 0.367 0.000
ptfairy 0.567 0.433 0.000 0.000 0.161 0.833 0.005 0.000 0.995 0.000 1.000 0.016 0.000
0.973 0.011 0.000 0.025 0.000 0.960 0.010 0.005 0.005 0.630 0.355 0.010
hobart 0.547 0.453 0.000 0.000 0.223 0.739 0.038 0.000 1.000 0.000 1.000 0.000 0.000
0.968 0.011 0.021 0.0435 0.000 0.9239 0.0163 0.0163 0.000 0.694 0.284 0.000
anxiousb 0.410 0.570 0.010 0.000 0.136 0.805 0.059 0.000 0.990 0.000 1.000 0.000 0.000
1.000 0.000 0.000 0.053 0.000 0.921 0.026 0.000 0.000 0.592 0.400 0.008

KEY : byronbay = Byron Bay, N.S.W. 25/5/'86 ; evanshead = Evans Head,N.S.W. 25/5/'86 ; yambal = Yamba, N.S.W. 7/6/'84. Total collection, divided as follows:- yambalge = large, fish > 17mm standard length, yambasml = small fish < 15mm standard length ; Subsequent collections : yamba2 = 22/5/'86, yamba3 = 23/5/'86 ; wooli = Wooli, N.S.W. 11/10/'85 ; nsolitary = North Solitary Island, N.S.W. 10/10/'85 ; coffsh1 = Coff's Harbour, N.S.W. 2/4/'85, coffsh2 = 21/5/'86 ; camden1 = Camden Heads, N.S.W. 2/10/'85, camden2 = South of Camden Heads 2/10/'85 ; forster1 = Forster, N.S.W. 1/10/'85, forster2 = 5/6/'85, forster3 = 20/5/'86 ; ptstephen = Port Stephens, N.S.W. 11/4/'85 ; newcastle = Newcastle, N.S.W. 1/4/'85 ; sydney = Sydney, N.S.W. 12/4/'85 ; jervisbay = Jervis Bay, N.S.W. 9/8/'84 ; eden = Eden, N.S.W. 22/6/'84 ; lakesent = Lakes Entrance, VIC. 18/6/'84 ; sanremo = Western Port Bay, VIC. 29/5/'84 ; ptlonsdal = Port Phillip Bay, VIC. 21/3/'85 ; cpatton1 = Cape Patton, VIC. 30/9/'85 , cpatton2 = west of Cape Patton, VIC. 30/9/'85 ; apollobay = Apollo Bay, VIC. 12/9/'85 ; ptfairy = Port Fairy, VIC. 11/'86 ; hobart = Hobart, TAS. 17/5/'84 ; anxiousb = Anxious Bay, S.A. 18/3/'86.

TABLE 7.2 : G Tests For *S. bassensis flindersi* Populations.

otu 1	vs	otu 2	g stat	d of f	prob.
byronbay		evanshead	16.9481	21	.7142
		yamba1	22.978	27	.6862
		yambalge	22.5329	20	.3123
		yambasml	18.7455	26	.8470
		yamba2	12.9239	18	.7961
		yamba3	9.04372	20	.9824
		wooli	18.6751	24	.7691
		nsolitary	22.5537	25	.6036
		coffsh1	12.754	18	.8060
		coffsh2	41.3021	18	.0014 *
		camden1	26.3238	24	.3369
		camden2	43.5102	23	.0060 *
		forster1	51.5335	23	.0006 *
		forster2	28.6238	22	.1559
		forster3	20.9954	19	.3371
		ptstephen	24.6295	21	.2636
		newcastle	17.9652	23	.7594
		sydney	21.0118	24	.6380
		jervisbay	15.8957	24	.8918
		eden	24.1906	23	.3933
		lakesent	22.3719	23	.4979
		sanremo	25.3378	23	.3331
		ptlonsdal	31.6486	23	.1077
		cpatton1	20.9407	22	.5244
		cpatton2	22.2568	22	.4447
		apollobay	29.959	24	.1861
		ptfairy	23.3757	23	.4390
		hobart	38.5488	22	.0159 *
		anxiousb	80.2698	21	0.000 *
evanshead		yamba1	31.4745	28	.2964
		yambalge	27.3133	21	.1607
		yambasml	26.6617	27	.4822
		yamba2	17.0155	21	.7102
		yamba3	13.2858	22	.9249
		wooli	15.1603	24	.9160
		nsolitary	19.8499	25	.7547
		coffsh1	23.9588	22	.3494
		coffsh2	51.1562	22	.0004 *
		camden1	22.5425	24	.5469
		camden2	44.9742	24	.0059 *
		forster1	50.5924	24	.0012 *
		forster2	26.7927	22	.2192
		forster3	20.494	21	.4902
		ptstephen	26.4722	24	.3297
		newcastle	19.9083	24	.7020
		sydney	19.7346	24	.7118
		jervisbay	22.0263	27	.7360
		eden	23.7053	22	.3629
		lakesent	14.1452	21	.8633
		sanremo	14.6236	21	.8414
		ptlonsdal	17.2388	21	.6965
		cpatton1	21.0138	24	.6379
		cpatton2	24.4087	21	.2737
		apollobay	22.7908	24	.5322

TABLE 7.2 (Cont.)

evanshead	ptfairy	22.6237	23	.4829
	hobart	21.7879	20	.3521
	anxiousb	64.9266	22	0.000 *
yambal	yambalge	19.5705	26	.8115
	yambasal	19.637	26	.8084
	yamba2	15.4742	26	.9480
	yamba3	23.2499	28	.7204
	wooli	40.3958	30	.0973
	nsolitary	53.3428	30	.0055 *
	coffsh1	20.0158	26	.7908
	coffsh2	52.5749	27	.0023 *
	camden1	29.3746	28	.3937
	camden2	72.217	29	0.000 *
	forster1	94.5015	28	0.000 *
	forster2	56.9944	28	.0010 *
	forster3	23.1273	27	.6782
	ptstephen	44.1188	27	.0201 *
	newcastle	29.9862	27	.3148
	sydney	19.2193	28	.8912
	jervisbay	25.6655	29	.6433
	eden	14.7063	27	.9734
	lakesent	31.1137	28	.3121
	sanremo	30.5627	28	.3368
	ptlonsdal	49.0082	28	.0083 *
	cpatton1	32.7155	27	.2066
	cpatton2	30.9788	27	.2720
	apollobay	36.3383	27	.1081
	ptfairy	42.4171	30	.0658
	hobart	52.4351	28	.0034 *
	anxiousb	99.4295	28	0.000 *
yambalge	yambasal	36.7305	25	.0612
	yamba2	15.8576	21	.7776
	yamba3	23.2116	21	.3328
	wooli	34.1004	23	.0637
	nsolitary	39.0932	26	.0478 *
	coffsh1	18.2165	18	.4415
	coffsh2	30.5797	20	.0610
	camden1	21.8448	25	.6447
	camden2	50.6173	22	.0005 *
	forster1	46.0771	23	.0029 *
	forster2	44.3474	23	.0048 *
	forster3	15.5396	18	.6246
	ptstephen	28.9855	22	.1453
	newcastle	30.842	21	.0763
	sydney	16.7619	23	.8208
	jervisbay	23.1357	25	.5696
	eden	18.8863	20	.5292
	lakesent	27.0578	23	.2535
	sanremo	23.4612	23	.4341
	ptlonsdal	47.2121	23	.0021 *
	cpatton1	31.151	22	.0931
	cpatton2	32.9184	22	.0630
	apollobay	28.9472	25	.2661
	ptfairy	23.9961	23	.4040

TABLE 7.2 (Cont.)

yambalge	hobart	39.37	21	.0089 *
	anxiousb	79.8372	22	0.000 *
yambasml	yamba2	21.2879	26	.7270
	yamba3	15.6481	27	.9593
	wooli	33.971	29	.2403
	nsolitary	43.6831	29	.0393 *
	coffsh1	23.6718	25	.5384
	coffsh2	73.9962	26	0.000 *
	camden1	39.2165	27	.0605
	camden2	51.4678	28	.0044 *
	forster1	74.405	27	0.000 *
	forster2	33.4604	27	.1823
	forster3	24.0844	26	.5711
	ptstephen	37.1372	26	.0727
	newcastle	33.2483	26	.1550
	sydney	30.2768	27	.3019
	jervisbay	30.8197	28	.3251
	eden	22.0378	26	.6866
	lakesent	38.423	27	.0714
	sanremo	40.0656	27	.0505
	ptlonsdal	44.5204	27	.0183 *
	cpatton1	32.1956	26	.1867
	cpatton2	42.3126	26	.0228 *
	apollobay	47.0229	27	.0098 *
	ptfairy	50.9545	29	.0071 *
	hobart	53.7927	27	.0016 *
	anxiousb	112.408	27	0.000 *
yamba2	yamba3	16.5402	22	.7882
	wooli	22.7411	26	.6475
	nsolitary	17.7936	26	.8832
	coffsh1	16.3177	19	.6360
	coffsh2	36.7173	20	.0126 *
	camden1	17.3686	24	.8325
	camden2	40.5217	25	.0258 *
	forster1	40.2452	23	.0144 *
	forster2	21.2348	22	.5063
	forster3	12.5553	19	.8605
	ptstephen	27.0025	22	.2111
	newcastle	24.0497	24	.4588
	sydney	17.82	25	.8499
	jervisbay	16.4877	26	.9237
	eden	21.7344	23	.5363
	lakesent	17.7611	23	.7703
	sanremo	16.3774	23	.8386
	ptlonsdal	23.7844	23	.4158
	cpatton1	24.3399	24	.4423
	cpatton2	23.1097	22	.3956
	apollobay	15.6614	23	.8694
	ptfairy	18.6082	24	.7725
	hobart	28.3642	22	.1639
	anxiousb	67.4051	23	0.000 *
yamba3	wooli	19.6745	24	.7151
	nsolitary	23.0151	26	.6321
	coffsh1	18.3547	21	.6265

TABLE 7.2 (Cont.)

yamba3	coffsh2	48.2155	21	.0006 *
	camden1	24.9723	25	.4639
	camden2	37.2755	24	.0411 *
	forster1	49.6829	25	.0023 *
	forster2	28.2576	24	.2493
	forster3	24.0974	23	.3984
	ptstephen	21.1186	24	.6317
	newcastle	20.2493	24	.6825
	sydney	19.4145	24	.7295
	jervisbay	15.9587	25	.9161
	eden	20.7419	24	.6539
	lakesent	23.2578	24	.5046
	sanremo	24.475	24	.4347
	ptlonsdal	34.5998	24	.0746
	cpatton1	16.4596	22	.7924
	cpatton2	30.6131	24	.1653
	apollobay	33.4968	26	.1482
	ptfairy	24.9654	24	.4076
	hobart	34.3308	23	.0605
	anxiousb	77.4943	22	0.000 *
wooli	nsolitary	28.8146	27	.3699
	coffsh1	26.2958	24	.3383
	coffsh2	68.1566	26	0.000 *
	camden1	32.0093	27	.2317
	camden2	52.8051	24	.0006 *
	forster1	81.1216	26	0.000 *
	forster2	34.4834	26	.1233
	forster3	15.8844	24	.8922
	ptstephen	32.8479	25	.1349
	newcastle	20.1765	25	.7375
	sydney	21.3495	26	.7237
	jervisbay	28.4671	27	.3872
	eden	28.0014	25	.3078
	lakesent	29.249	26	.2999
	sanremo	31.5099	26	.2098
	ptlonsdal	33.4166	26	.1504
	cpatton1	29.0053	25	.2637
	cpatton2	36.1247	26	.0894
	apollobay	36.5586	29	.1579
	ptfairy	33.9279	25	.1095
	hobart	46.927	25	.0050 *
	anxiousb	94.0639	25	0.000 *
nsolitary	coffsh1	35.9815	27	.1157
	coffsh2	59.0225	26	.0002 *
	camden1	34.8738	26	.1144
	camden2	54.1277	26	.0010 *
	forster1	85.9809	29	0.000 *
	forster2	39.5491	26	.0432 *
	forster3	20.5692	25	.7164
	ptstephen	44.2955	27	.0193 *
	newcastle	37.7439	27	.0820
	sydney	25.5006	28	.6005
	jervisbay	27.5794	29	.5405
	eden	48.9579	27	.0060 *

TABLE 7.2 (Cont.)

nsolitary	lakesent	30.7194	27	.2828
	sanremo	28.6189	27	.3796
	ptlonsdal	37.4864	27	.0863
	cpatton1	30.3114	27	.3003
	cpatton2	43.6284	26	.0166 *
	apollobay	38.3172	27	.0730
	ptfairy	25.3594	27	.5543
	hobart	37.2412	25	.0548
	anxiousb	91.7458	27	0.000 *
coffsh1	coffsh2	33.3492	20	.0309 *
	camden1	29.146	26	.3045
	camden2	55.2758	22	.0001 *
	forster1	48.5473	21	.0006 *
coffsh1	forster2	37.2816	23	.0304 *
	forster3	16.7946	17	.4684
	ptstephen	27.8246	21	.1452
	newcastle	22.61	23	.4837
	sydney	19.7138	24	.7129
	jervisbay	15.7564	24	.8967
	eden	18.8326	20	.5327
	lakesent	18.6938	23	.7188
	sanremo	21.1605	23	.5713
	ptlonsdal	32.1251	23	.0976
	cpatton1	22.3329	22	.4402
	cpatton2	22.8464	22	.4104
	apollobay	35.2374	26	.1066
	ptfairy	20.8092	23	.5927
	hobart	42.9299	22	.0048 *
	anxiousb	74.8161	20	0.000 *
coffsh2	camden1	39.3148	24	.0253 *
	camden2	80.0569	25	0.000 *
	forster1	68.4352	25	0.000 *
	forster2	80.971	23	0.000 *
	forster3	39.1192	20	.0064 *
	ptstephen	38.6001	22	.0157 *
	newcastle	34.8381	22	.0403 *
	sydney	38.4478	24	.0312 *
	jervisbay	45.1523	26	.0113 *
	eden	53.6841	24	.0005 *
	lakesent	50.8696	24	.0011 *
	sanremo	49.2956	24	.0017 *
	ptlonsdal	60.9623	24	0.000 *
	cpatton1	47.8221	22	.0011 *
	cpatton2	47.8095	22	.0011 *
	apollobay	47.6047	23	.0019 *
	ptfairy	50.0643	24	.0014 *
	hobart	69.9918	23	0.000 *
	anxiousb	80.8802	21	0.000 *
camden1	camden2	56.7012	26	.0005 *
	forster1	68.8132	26	0.000 *
	forster2	39.0561	24	.0269 *
	forster3	22.184	25	.6251

TABLE 7.2 (Cont.)

camden1	ptstephen	32.8904	25	.1338
	newcastle	27.0533	25	.3532
	sydney	24.0997	26	.5703
	jervisbay	28.9608	28	.4145
	eden	28.6734	26	.3261
	lakesent	24.7017	25	.4792
	sanremo	24.0481	25	.5166
	ptlonsdal	35.6964	25	.0763
	cpatton1	42.9294	26	.0196 *
	cpatton2	30.7431	24	.1613
	apollobay	19.8561	25	.7543
	ptfairy	34.231	27	.1594
	hobart	40.5501	24	.0187 *
	anxiousb	96.2522	26	0.000 *
camden2	forster1	24.316	25	.5012
	forster2	41.5014	25	.0203 *
	forster3	39.3945	23	.0180 *
	ptstephen	44.7538	25	.0089 *
	newcastle	58.141	25	.0002 *
	sydney	62.7517	27	.0001 *
	jervisbay	55.2083	27	.0011 *
	eden	60.1245	23	0.000 *
	lakesent	66.131	25	0.000 *
	sanremo	61.1403	25	.0001 *
	ptlonsdal	63.451	25	0.000 *
	cpatton1	66.893	26	0.000 *
	cpatton2	69.6719	24	0.000 *
	apollobay	63.2197	28	.0002 *
	ptfairy	53.2307	24	.0005 *
	hobart	73.5051	24	0.000 *
	anxiousb	98.0499	24	0.000 *
forster1	forster2	51.5074	23	.0006 *
	forster3	38.4897	22	.0161 *
	ptstephen	43.3832	24	.0090 *
	newcastle	62.595	26	.0001 *
	sydney	72.7492	26	0.000 *
	jervisbay	64.4985	26	0.000 *
	eden	75.0528	23	0.000 *
	lakesent	68.8866	24	0.000 *
	sanremo	62.5433	24	0.000 *
	ptlonsdal	72.258	24	0.000 *
	cpatton1	91.6026	27	0.000 *
	cpatton2	85.7499	24	0.000 *
	apollobay	68.9598	27	0.000 *
	ptfairy	72.021	24	0.000 *
	hobart	100.414	24	0.000 *
	anxiousb	136.526	24	0.000 *
forster2	forster3	14.8274	21	.8315
	ptstephen	47.8846	25	.0039 *
	newcastle	49.155	26	.0040 *
	sydney	43.0977	26	.0189 *
	jervisbay	48.9391	28	.0085 *
	eden	46.8869	24	.0035 *

TABLE 7.2 (Cont.)

forster2	lakesent	29.8458	23	.1539
	sanremo	29.0206	23	.1796
	ptlonsdal	33.3008	23	.0760
	cpatton1	64.5966	27	.0001 *
	cpatton2	48.4762	23	.0015 *
	apollobay	31.5918	24	.1374
	ptfairy	46.9352	24	.0034 *
	hobart	43.6021	22	.0040 *
	anxiousb	114.975	25	0.000 *
forster3	ptstephen	24.5739	21	.2661
	newcastle	24.9615	22	.2989
	sydney	11.564	24	.9844
	jervisbay	27.4754	26	.3847
	eden	17.0336	21	.7091
	lakesent	19.6881	23	.6606
	sanremo	18.638	23	.7220
	ptlonsdal	25.3154	23	.3342
	cpatton1	30.3673	24	.1729
	cpatton2	25.3477	22	.2807
	apollobay	20.1421	24	.6887
	ptfairy	18.6588	23	.7208
	hobart	27.3104	21	.1608
	anxiousb	63.418	22	0.000 *
ptstephen	newcastle	10.1495	22	.9849
	sydney	28.5617	24	.2371
	jervisbay	32.8408	27	.2024
	eden	35.0615	24	.0675
	lakesent	43.7479	26	.0161 *
	sanremo	42.7427	26	.0206 *
	ptlonsdal	50.4145	26	.0028 *
	cpatton1	32.4887	23	.0904
	cpatton2	45.3523	24	.0053 *
	apollobay	38.7849	26	.0511
	ptfairy	52.489	26	.0016 *
	hobart	70.7567	25	0.000 *
	anxiousb	101.371	23	0.000 *
newcastle	sydney	20.8796	24	.6458
	jervisbay	27.4117	27	.4418
	eden	27.9933	24	.2603
	lakesent	32.7637	26	.1691
	sanremo	36.0725	26	.0903
	ptlonsdal	37.1193	26	.0729
	cpatton1	21.5988	23	.5446
	cpatton2	28.3832	24	.2442
	apollobay	29.2146	26	.3014
	ptfairy	44.4297	26	.0136 *
	hobart	57.5759	25	.0002 *
	anxiousb	85.6958	24	0.000 *
sydney	jervisbay	24.8662	28	.6351
	eden	13.4518	25	.9705
	lakesent	19.778	25	.7584
	sanremo	20.6355	25	.7127

TABLE 7.2 (Cont.)

sydney	ptlonsdal	34.1756	25	.1042
	cpatton1	19.0677	24	.7484
	cpatton2	27.7796	25	.3181
	apollobay	27.7366	26	.3715
	ptfairy	20.4851	27	.8098
	hobart	30.5398	24	.1675
	anxiousb	77.5766	24	0.000 *
jervisbay	eden	32.3996	27	.2176
	lakesent	24.7097	27	.5907
	sanremo	21.9877	27	.7380
	ptlonsdal	35.2549	27	.1325
	cpatton1	17.0819	26	.9066
	cpatton2	41.1898	28	.0516
	apollobay	38.6472	30	.1336
	ptfairy	25.7676	26	.4759
	hobart	49.5186	27	.0052 *
	anxiousb	88.1561	26	0.000 *
eden	lakesent	26.4786	23	.2788
	sanremo	27.6556	23	.2291
	ptlonsdal	41.8623	23	.0094 *
	cpatton1	32.2969	25	.1496
	cpatton2	29.15	22	.1406
	apollobay	37.3987	26	.0688
	ptfairy	30.2723	24	.1759
	hobart	37.1889	22	.0226 *
	anxiousb	86.5743	23	0.000 *
lakesent	sanremo	2.11312	21	*1.0000
	ptlonsdal	18.7723	21	.5997
	cpatton1	35.2627	26	.1061
	cpatton2	24.6134	22	.3159
	apollobay	22.0256	25	.6343
	ptfairy	19.5194	24	.7238
	hobart	17.5315	21	.6784
	anxiousb	87.916	23	0.000 *
sanremo	ptlonsdal	17.758	21	.6643
	cpatton1	35.1082	26	.1093
	cpatton2	26.639	22	.2253
	apollobay	18.5638	25	.8175
	ptfairy	17.4429	24	.8292
	hobart	15.9272	21	.7737
	anxiousb	77.1517	23	0.000 *
ptlonsdal	cpatton1	44.3388	26	.0139 *
	cpatton2	33.5522	22	.0545
	apollobay	23.3505	25	.5571
	ptfairy	32.7583	24	.1093
	hobart	32.8612	21	.0478 *
	anxiousb	83.8972	23	0.000 *

TABLE 7.2 (Cont.)

cpatton1	cpatton2	42.7271	25	.0150 *
	apollobay	49.3832	27	.0054 *
	ptfairy	36.8809	27	.0973
	hobart	49.7311	25	.0023 *
	anxiousb	82.0976	23	0.000 *
cpatton2	apollobay	27.2248	24	.2941
	ptfairy	34.482	24	.0765
	hobart	43.5128	22	.0041 *
	anxiousb	70.6749	22	0.000 *
apollobay	ptfairy	41.4117	28	.0492 *
	hobart	33.0131	24	.1038
	anxiousb	77.6002	25	0.000 *
ptfairy	hobart	35.2659	24	.0646
	anxiousb	79.9433	24	0.000 *
hobart	anxiousb	81.12	23	0.000 *

TABLE 7.3 : Gene Frequency Input Data For The Loci Gpi-1, Gpi-2, Gpi-3, Aat-2 and PepC of *S. bassensis bassensis* From Four Localities in Southern Australia.

	4	5	B						
3	3	3	2	3					
STVINGULF	0.039	0.950	0.039	0.944	0.005	0.978	0.994	0.250	0.644
SPENGULF	0.000	1.000	0.000	1.000	0.000	1.000	1.000	0.012	0.860
KANGARIS	0.035	0.953	0.035	0.942	0.000	1.000	1.000	0.006	0.956
MANDURAH	0.010	0.985	0.015	0.970	0.010	0.990	1.000	0.006	0.956

KEY : STVINGULF = Saint Vincent's Gulf, S.A. 11/6/'84 ; SPENGULF = Spencer Gulf, S.A. 5/'84 ; KANGARIS = Kangaroo Island, S.A. 1/6/'84 ; MANDURAH = Mandurah, W.A. 13/5/'85.

TABLE 7.4 : Gene Frequency Input Data For The Loci Gpi-1, Gpi-2, Gpi-3, Idh-1, Mpi, Pgm-1 and Sdh of *S. robusta* From Six Localities in New South Wales.

	7	7	b						
2	3	3	2	5	5	3			
BYRONBY	0.993	0.007	0.987	0.007	0.987	0.286	0.007	0.000	
0.987	0.000	0.007	0.355	0.579	0.059	0.295	0.610		
EVANSHD	1.000	0.014	0.973	0.007	0.980	0.288	0.014	0.020	
0.966	0.000	0.000	0.493	0.419	0.081	0.194	0.629		
YAMBA	1.000	0.000	0.983	0.000	0.983	0.298	0.000	0.000	
0.991	0.009	0.000	0.246	0.622	0.132	0.208	0.583		
SANDON	0.995	0.006	0.989	0.000	0.990	0.239	0.000	0.000	
1.000	0.000	0.000	0.283	0.622	0.094	0.130	0.652		
FORST1	1.000	0.000	0.993	0.000	0.993	0.172	0.000	0.000	
1.000	0.000	0.000	0.318	0.538	0.144	0.229	0.610		
FORST2	0.995	0.005	0.986	0.005	0.995	0.193	0.000	0.000	
1.000	0.000	0.000	0.356	0.574	0.069	0.224	0.619		
COFFSH	0.989	0.006	0.966	0.000	0.983	0.224	0.007	0.000	
0.993	0.000	0.000	0.369	0.506	0.125	0.207	0.627		

KEY : BYRONBAY = Byron Bay 25/5/'86 ; EVANSHEAD = Evan's Head 25/5/'86 ; YAMBA = Yamba 25/5/'86 ; SANDON = Sandon Bluff 5/6/'85 ; FORST1 = Forster 1/10/'85 ; FORST2 = Forster 20/5/'86 ; COFFSH = Coff's Harbour 26/3/'85.

TABLE 7.5 : Gene Frequency Input Data For The Loci Gpt, Gpi-1, Gpi-2, Gpi-3, Pgm-1 and Pgm-2 of *S. punctata* From Three Localities In South Australia.

	4		6 b							
3	2	2	3	3	4					
spencer1	0.025	0.500	0.020	0.022	0.020	0.010	0.021	0.978	0.010	0.970	0.020
spencer2	0.000	0.906	0.014	0.000	0.000	0.000	0.000	1.000	0.030	0.955	0.015
angusin	0.000	0.618	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.991	0.000
kangaroo	0.091	0.114	0.000	0.000	0.000	0.000	0.026	0.947	0.019	0.904	0.038

KEY : spencer1 = Upper Spencer Gulf 1/11/'85 ; spencer2 = Upper Spencer Gulf 3/11/'85 ; angusin = Angus Inlet 13/2/86 ; kangaroo = Kangaroo Island 20/11/'85.

TABLE 7.6 : Gene Frequency Input Data For The Loci Gpt, Gpi-1, Gpi-2, Gpi-3 and Pgm-1 of *S. punctata* From Four Localities In South Australia.

	5		5 b					
3	2	2	3	3				
adelaide	0.000	0.353	0.000	0.000	0.000	0.000	0.000	1.000
spencer1	0.025	0.500	0.020	0.022	0.020	0.010	0.021	0.978
spencer2	0.000	0.906	0.014	0.000	0.000	0.000	0.000	1.000
angusin	0.000	0.618	0.000	0.000	0.000	0.000	0.000	1.000
kangaroo	0.091	0.114	0.000	0.000	0.000	0.000	0.026	0.947

KEY : adelaide = Port Adelaide 13/9/'84 ; spencer1 = Upper Spencer Gulf 1/11/'85 ; spencer2 = Upper Spencer Gulf 3/11/'85 ; angusin = Angus Inlet 13/2/86 ; kangaroo = Kangaroo Island 20/11/'85.

TABLE 7.7 : Gene Frequency Input Data For The Loci Gpt, Gpi-1, Gpi-2 and Gpi-3 of *S. punctata* From Five Localities In Southern Australia.

	6		4 b			
3	2	2	3			
adelaide	0.000	0.353	0.000	0.000	0.000	0.000
spencer1	0.025	0.500	0.020	0.022	0.020	0.010
spencer2	0.000	0.906	0.014	0.000	0.000	0.000
angusin	0.000	0.618	0.000	0.000	0.000	0.000
kangaroo	0.091	0.114	0.000	0.000	0.000	0.000
cornerin	0.000	0.054	0.020	0.007	0.000	0.000

KEY : adelaide = Port Adelaide 13/9/'84 ; spencer1 = Upper Spencer Gulf 1/11/'85 ; spencer2 = Upper Spencer Gulf 3/11/'85 ; angusin = Angus Inlet 13/2/86 ; kangaroo = Kangaroo Island 20/11/'85 ; cornerin = Corner Inlet, VIC. 13/4/'85.

APPENDIX 8 : DESCRIPTIVE STATISTICS FOR RATIO, LG-RATIO AND ALLOM.

KEY : VAR. = variable acronym (see text); x = mean; Var. = variance;
C.L. = 95% confidence limits about mean value.

TABLE 8.1 : Summary of descriptive statistics for percentage RATIO shape variates by geographical area.

	YAMBA			MANDURAH			HOBART			EDEN			SPENCER GULF		
VAR.	x	Var.	C.L.	x	Var.	C.L.	x	Var.	C.L.	x	Var.	C.L.	x	Var.	C.L.
HDSL	27.19	0.474	0.137	26.86	0.685	0.168	26.53	0.513	0.142	27.49	0.437	0.131	27.56	0.351	0.118
FDSL	34.46	0.912	0.189	32.90	1.083	0.210	34.17	0.635	0.158	34.86	0.654	0.161	33.03	0.399	0.126
SDSL	55.59	0.936	0.192	53.51	1.990	0.286	55.47	1.103	0.209	56.33	0.819	0.18	54.04	0.587	0.153
ANSL	54.07	1.050	0.192	53.59	2.800	0.339	54.08	1.154	0.213	54.71	0.971	0.195	54.02	0.688	0.166
CASL	7.17	0.111	0.066	6.79	0.120	0.070	6.40	0.089	0.060	6.54	0.101	0.063	5.61	0.065	0.051
HEHL	22.23	0.756	0.172	20.04	0.714	0.171	22.42	0.646	0.160	21.80	0.626	0.157	19.67	0.446	0.133
EYHL	25.01	1.190	0.216	26.17	5.791	0.488	24.89	1.706	0.259	25.04	1.568	0.249	26.54	2.025	0.284
SNHL	42.13	2.691	0.326	41.90	3.345	0.371	43.27	1.303	0.227	43.10	1.406	0.236	43.88	2.590	0.321

TABLE 8.2 : Summary of descriptive statistics for LGRATIO shape variates by geographical area.

	YAMBA			MANDURAH			HOBART			EDEN			SPENCER GULF		
VAR.	X	Var.	CL	X	Var.	CL	X	Var.	CL	X	Var	CL	X	Var.	CL
HDSL	-0.57	0	0.002	-0.57	0	0.003	-0.58	0	0.003	-0.56	0	0.002	-0.56	0	0.002
FDSL	-0.46	0	0.002	-0.48	0	0.003	-0.47	0	0.002	-0.46	0	0.002	-0.48	0	0.002
SDSL	-0.26	0	0.002	-0.27	0	0.002	-0.26	0	0.002	-0.25	0	0.002	-0.27	0	0.002
ANSL	-0.27	0	0.002	-0.27	0	0.003	-0.27	0	0.002	-0.26	0	0.002	-0.27	0	0.002
CASL	-1.15	0	0.004	-1.17	0.001	0.004	-1.19	0	0.004	-1.19	0	0.004	-1.25	0	0.004
HEHL	-0.65	0	0.004	-0.70	0	0.004	-0.65	0	0.003	-0.66	0	0.003	-0.71	0	0.003
EYHL	-0.60	0	0.004	-0.58	0.002	0.008	-0.61	0.001	0.005	-0.60	0	0.004	-0.58	0.001	0.005
SNHL	-0.37	0.002	0.008	-0.38	0	0.004	-0.36	0	0.002	-0.37	0	0.003	-0.36	0	0.003

TABLE 8.3 : Summary of descriptive statistics for ALLOM shape variates by geographical area.

	YAMBA			MANDURAH			HOBART			EDEN			SPENCER GULF		
VAR.	X	Var.	CL	X	Var.	CL	X	Var.	CL	X	Var.	CL	X	Var.	CL
HDSL	1.62	0	0.002	1.62	0	0.003	1.61	0	0.003	1.63	0	0.002	1.62	0	0.002
FDSL	1.73	0	0.003	1.71	0	0.003	1.72	0	0.002	1.73	0	0.002	1.71	0	0.002
SDSL	1.94	0	0.002	1.92	0	0.003	1.93	0	0.002	1.94	0	0.002	1.92	0	0.001
ANSL	1.93	0	0.002	1.92	0	0.003	1.92	0	0.002	1.93	0	0.002	1.92	0	0.001
CASL	1.05	0	0.004	1.02	0	0.005	1.00	0	0.004	1.01	0	0.005	0.94	0	0.004
HEHL	0.97	0	0.004	0.92	0	0.004	0.98	0	0.003	0.97	0	0.004	0.92	0	0.003
EYHL	1.02	0	0.003	1.01	0.001	0.007	1.04	0	0.004	1.04	0	0.003	1.04	0	0.004
SNHL	1.26	0.002	0.009	1.26	0	0.003	1.26	0	0.002	1.26	0	0.003	1.28	0	0.003

APPENDIX 9 : EFFECTS OF VARIOUS TRANSFORMATIONS ON THE NORMALITY OF VARIABLES.

KEY : VAR.= variable acronym (see text); G_1 = skewness; G_2 = kurtosis;
SIG.= significance of G_1 and G_2 (* = $p<0.05$;
** = $p<0.01$; *** = $p<0.001$; - = nonsignificant).

TABLE 9.1 : Normality of RATIO shape variates by geographical area.

	YAMBA				MANDURAH				HOBART			
VAR.	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.
HDSL	0.032	-	-0.476	-	0.379	-	0.908	-	-0.123	-	-0.154	-
FDSL	-0.744	**	2.297	***	0.776	**	1.203	*	0.355	-	-0.497	-
SDSL	0.175	-	-0.186	-	-0.072	-	1.736	***	-0.051	-	-0.172	-
ANSL	0.096	-	-0.378	-	0.763	*	2.819	***	0.146	-	0.021	-
CASL	-0.158	-	-0.256	-	-0.432	-	0.329	-	0.258	-	0.032	-
HEHL	0.403	-	-0.168	-	0.128	-	-0.741	-	-0.041	-	0.063	-
EYHL	0.161	-	-0.276	-	0.678	**	0.526	-	-0.298	-	0.118	-
SNHL	-0.154	-	0.285	-	-0.383	-	-0.320	-	0.128	-	0.763	-

	EDEN				SPENCER GULF			
G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.
0.585	*	1.849	***	-0.132	-	-0.156	-
0.474	-	2.213	***	-0.271	-	0.041	-
2.064	***	9.914	***	-0.034	-	-0.362	-
0.828	***	2.612	***	-0.254	-	-0.351	-
0.090	-	0.453	-	0.082	-	-0.118	-
0.010	-	0.250	-	-0.306	-	0.738	-
0.199	-	0.118	-	-0.046	-	0.160	-
0.070	-	-0.247	-	-0.149	-	0.948	-

TABLE 9.2 : Normality of LGRATIO shape variates by geographical area.

	YAMBA				MANDURAH				HOBART			
VAR.	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.
HDSL	-0.025	-	-0.458	-	0.252	-	0.958	-	-0.197	-	-0.046	-
FDSL	-0.900	***	2.715	***	0.659	**	0.977	*	0.308	-	-0.521	-
SDSL	0.130	-	-0.222	-	-0.219	-	1.957	***	-0.102	-	-0.154	-
ANSL	0.051	-	-0.371	-	0.570	*	2.667	***	0.087	-	0.031	-
CASL	-0.276	-	-0.204	-	-0.598	*	0.573	-	0.122	-	0.036	-
HEHL	0.308	-	-0.265	-	0.051	-	-0.774	-	-0.149	-	0.066	-
EYHL	0.051	-	-0.268	-	0.415	-	0.135	-	-0.459	-	0.319	-
SNHL	7.591	***	69.225	***	-0.483	-	-0.223	-	0.024	-	0.650	-

	EDEN				SPENCER GULF			
	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.
	0.465	-	1.607	**	-0.189	-	-0.148	-
	0.346	-	1.821	***	-0.324	-	0.000	-
	1.897	***	8.745	***	-0.068	-	-0.339	-
	0.729	**	2.250	***	-0.290	-	-0.333	-
	-0.840	-	0.451	-	-0.045	-	-0.080	-
	-0.108	-	0.183	-	-0.439	-	0.869	-
	0.051	-	-0.012	-	-0.219	-	0.279	-
	-0.002	-	-0.204	-	-0.303	-	0.916	-

TABLE 9.3 : Normality of ALLOM shape variates by geographical area.

	YAMBA				MANDURAH				HOBART			
VAR.	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.
HDSL	-0.145	-	-0.367	-	0.321	-	1.071	*	-0.457	-	0.276	-
FDSL	-0.884	***	2.599	***	0.662	**	0.971	*	0.238	-	0.291	-
SDSL	0.135	-	-0.569	-	-0.431	-	2.055	***	-0.289	-	-0.003	-
ANSL	0.070	-	-0.365	-	0.492	*	2.537	***	-0.018	-	0.486	-
CASL	-0.279	-	-0.201	-	-0.612	*	0.479	-	0.140	-	0.113	-
HEHL	0.326	-	-0.239	-	0.026	-	-0.267	-	-0.081	-	-0.092	-
EYHL	0.170	-	-0.108	-	0.179	-	1.937	***	0.034	-	-0.185	-
SNHL	7.636	***	69.811	***	-0.488	-	0.655	-	0.043	-	1.027	*

	EDEN				SPENCER GULF			
	G_1	SIG.	G_2	SIG.	G_1	SIG.	G_2	SIG.
	0.465	-	1.629	***	-0.143	-	-0.156	-
	0.363	-	1.675	***	-0.304	-	0.110	-
	1.995	***	8.808	***	0.062	-	-0.211	-
	0.607	*	2.005	***	-0.155	-	-0.606	-
	-0.137	-	0.269	-	-0.047	-	-0.100	-
	-0.108	-	0.083	-	-0.708	**	1.501	**
	0.533	*	0.931	-	-0.206	-	0.734	-
	0.233	-	-0.293	-	-0.093	-	0.379	-

APPENDIX 10 : STATISTICS FOR SIMPLE LINEAR REGRESSION OF SIZE ON SHAPE.

KEY : Var. = variable acronym (see text); Sig. = significance of slope
(* = p<0.05; ** = p<0.01; *** = p<0.001; - = nonsignificant);
R^2 = squared correlation coefficient.

TABLE 10.1 : Efficacy of size removal for the RATIO method by geographical area.

	Yamba		Mandurah		Hobart		Eden		Spencer Gulf	
Var.	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2
HDSL	***	0.18	-	0.01	***	0.13	-	0.00	***	0.16
FDSL	-	0.00	-	0.00	***	0.18	-	0.02	**	0.09
SDSL	*	0.05	**	0.06	***	0.14	**	0.06	-	0.02
ANSL	-	0.00	-	0.01	***	0.17	***	0.12	-	0.02
CASL	-	0.00	**	0.06	-	0.00	-	0.00	-	0.00
HEHL	-	0.01	***	0.20	***	0.10	-	0.01	**	0.08
EYHL	***	0.22	***	0.43	***	0.24	***	0.53	***	0.28
SNHL	-	0.01	***	0.44	***	0.10	***	0.13	***	0.34

TABLE 10.2 : Efficacy of size removal for the LGRATIO method by geographical area.

	Yamba		Mandurah		Hobart		Eden		Spencer Gulf	
Var.	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2
HDSL	***	0.18	-	0.01	***	0.13	-	0.00	***	0.16
FDSL	-	0.00	-	0.00	***	0.18	-	0.02	***	0.09
SDSL	*	0.05	*	0.05	***	0.14	**	0.07	-	0.02
ANSL	-	0.00	-	0.01	***	0.17	***	0.12	-	0.02
CASL	-	0.00	**	0.06	-	0.00	-	0.00	-	0.00
HEHL	-	0.01	***	0.20	***	0.11	-	0.01	**	0.08
EYHL	***	0.22	***	0.43	***	0.25	***	0.54	***	0.28
SNHL	-	0.01	***	0.44	***	0.10	***	0.13	***	0.34

TABLE 10.3: Efficacy of size removal for the ALLOM method by geographical area.

	Yamba		Mandurah		Hobart		Eden		Spencer Gulf	
Var.	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2
HDSL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
FDSL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
SDSL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
ANSL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
CASL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
HEHL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
EYHL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
SNHL	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00