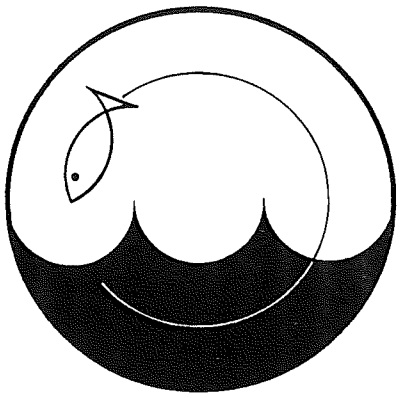
# GENETIC STRUCTURE OF OCEAN JACKET POPULATIONS - PILOT STUDY

(Final Report, FIRDC 91/26)

P. I. Dixon and J. Musa



CENTRE FOR MARINE SCIENCE

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## Acknowledgments

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## Summary and Recommendations

1. Forty four enzymes were surveyed for genetic variation using horizontal starch gel electrophoresis. Twelve enzymes showed no activity. Of the remaining 32, which represented 38 presumed loci, eleven were found to be polymorphic, viz *AAT-1*, ADA*, EST-1*, G-6PDH*, GPI*, IDH*, LDH*, MDH*, ME*, PEP (leu-tyr)** and *PGM**.

2. Nine of these polymorphic loci were used to compare samples collected from three localities: Coffs Harbour, New South Wales, Port Lincoln area, South Australia, and the Great Australian Bight, S.A. *ADA** and *ME** were omitted from these comparisons because they did not meet the consistency criteria of Shaklee and Keenan (1986).

3. Some evidence of population sub-structure was found. However the sample from Port Lincoln was more closely related to that from Coffs Harbour than it was to that from the Great Australian Bight. This finding was also weakly supported by morphometric and meristic comparisons made between those samples by Musa (1994).

4. This pilot study indicates that there is sufficient polymorphism for a detailed study of the population structure of ocean jackets to be feasible.

5. Further study should include isozyme electrophoresis of the enzymes already found to be polymorphic. These should include comparisons of samples from a greater number of sites, including Western Australian waters. The sampling regime should follow that suggested by Richardson *et al.* (1986) or be similar so that it includes estimates of variation both in time and space.

6. Other biochemical approaches eg mtDNA analysis are also recommended to give a more detailed understanding of the structure of the ocean jacket population(s). Such information is essential to the appropriate biological management of any species.

## Introduction

Ocean jackets (*Nelusetta ayraudi*) are distributed from North West Cape (W.A.) through southern coastal waters to the south of Queensland. They have not been recorded from Tasmanian waters. Seawards their distribution ranges from coastal embayments where juveniles school seasonally to waters just off the edge of the continental shelf (Hutchins and Thompson, 1983). They mainly inhabit the mid-water bottom habitat over either sandy bottom (S.A.) or reef areas (N.S.W.). There are indications from studies in South Australia that ocean jackets may leave the continental shelf to spawn in offshore waters (Grove-Jones and Burnell, 1991).

In the 1940's and 50's there was an intensive trap fishery in N.S.W. This fishery suffered a dramatic decline and was subsequently replaced by a snapper trap fishery in the same area. Some ocean jackets are still taken in N.S.W. but the catch, which is probably less that 100 tonnes p.a., is difficult to estimate because most of it is sold to local markets.

More recently a commercial trap fishery developed in South Australia. The catch rose rapidly to almost 900 tonnes in 1988/89 (Anon., 1989). Since then the catch has levelled out but ocean jackets remain one of the largest marine scale fisheries (by weight) in South Australia.

Given the rapid success of the ocean jacket fishery in South Australia and the good eating qualities of the flesh, expansion of the fishery into other areas is a possibility. The past dramatic collapse of the N.S.W. fishery, the recent flattening off of the catch in South Australia and the suggested cohesion of the schools (Grove-Jones and Burnell, 1990) raise questions about the genetic stock structure of the ocean jacket population(s) in Australia.

This study examines the genetics of *N. ayraudi* as revealed by isozyme analysis. It investigates the feasibility of carrying out a full scale project aimed at determining whether the ocean jackets in Australian waters belong to one large interbreeding population or whether two or more discrete genetic stocks exist. This information is crucial to biologically appropriate management of the species.

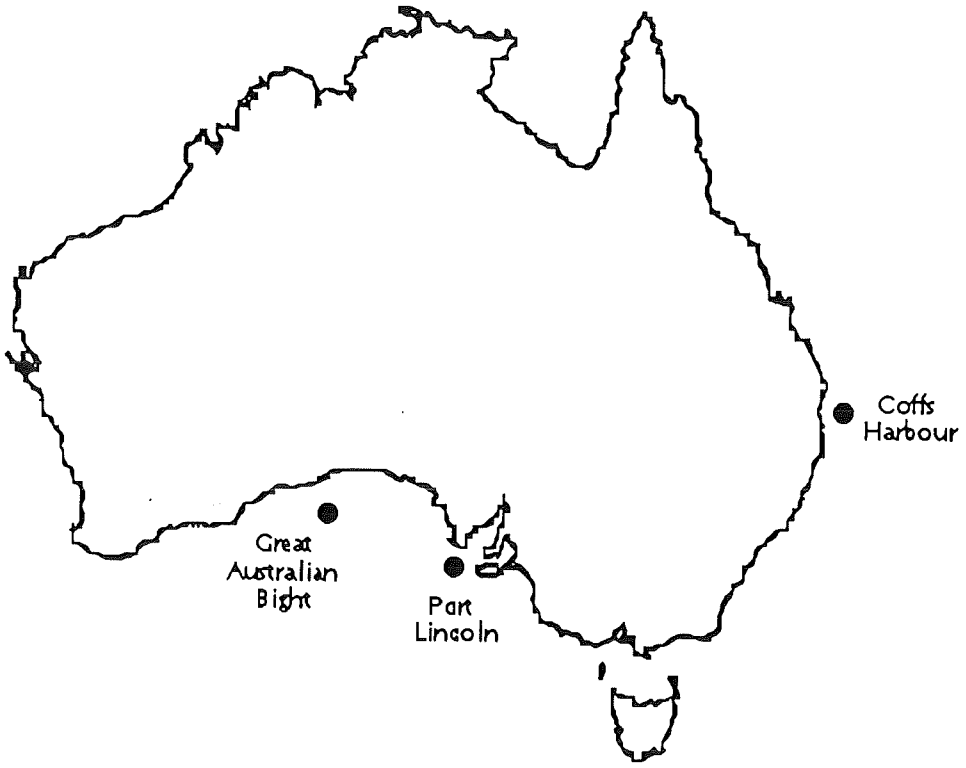



Figure 1: Map showing the collection sites for ocean jackets.

Table 1: Collection data and some key characters for three populations of ocean jackets. Key: M=Male; F=Female; LCF = length to caudal fork.

| Population | Collection Date | # of Samples | Sex Ratio (M:F) | Size Range (LCF mm) |
|---|---|---|---|---|
| PL (Port Lincoln, SA) | Feb.92 | 89 | 36:53 | 272-522 (320) |
| GAB (Great Aust. Bight, SA) | Mar.92 | 82 | 32:50 | 265-465 (307) |
| CH (Coffs Harbour, NSW) | July 92 | 95 | 46:49 | 225-520 (372) |

## Methods

### Sample collection

Collections of approximately 90 fish each were obtained from two localities in S.A. and one in N.S.W. The details of these collections are shown in Table 1 and Figure 1.

Fish were received frozen and kept at -20°C in the laboratory. Samples of liver, muscle and heart were taken from partially thawed fish and store at -70°C until required for electrophoresis.

### Electrophoresis

Full details of the electrophoretic procedures used in this study follow the methods described in Dixon *et al.*, 1987. Starch gels were made from 11%(w/v) Electrostarch (Lot no. 89).

Forty four enzymes were surveyed for genetic variation using horizontal starch gel electrophoresis for three different tissues (heart, liver and white muscle). Tissue samples were homogenised in an equal volume of homogenising buffer (10mg NADP, 100ml $H_2O$, 0.001M β-mercaptomethanol, 0.01M EDTA at pH 7.5) using a perspex rod. Details of enzymes surveyed for genetic variation are found in Appendix 1 and electrophoresis running conditions and presumed number of loci for each tissue are listed in Appendix 2.

Twelve enzymes showed no activity. Of the remaining 32, which represented 38 presumed loci, eleven were found to be polymorphic, viz *AAT-1*, ADA*, EST-1*, G-6PDH*, GPI-1*, IDH*, LDH*, MDH*, ME*, PEP*(leu-tyr)* and *PGM**. Only those loci with patterns of variation that were consistent with the known sub-unit structure of the enzyme (Shaklee and Keenan, 1986) and/or displayed a phenotype distribution in Hardy-Weinberg equilibrium were used for the population analysis. Ada and Me did not meet these criteria. Thus nine loci were used in the detailed analyses and sample comparisons.

### Data analysis

Names of enzymes and Enzyme Commission (EC) numbers follow the recommendations of the Commission of Biochemical Nomenclature (Anon., 1984). Abbreviated names of enzymes, loci and alleles follow the recommendations of Shaklee *et al.* (1990). For multilocus enzymes the form with the most anodal migration was designated 1. For each locus the alleles were

designated alphabetically with the most anodally-migrating allele designated "a". The putative genotype data were tabulated as allele frequency distributions. The data was organised into Datyp-1 format (single individual genotypes in alphabetic characters) of BIOSYS-1 computer program (Swofford and Selander 1989). Tests for Hardy-Weinberg equilibrium, polymorphism (P) and heterozygosity (H), contingency chi-square tests, Wright's F-statistics, different measures of genetic identity (I) and distance (D) indices and UPGMA and Distance Wagner clustering were calculated using the same computer program.

Deviations from Hardy-Weinberg proportions were tested for each polymorphic locus in each population. In cases of polymorphic loci examined, where more than two alleles are present in a population sample pooled genotypes were used for all subsequent analyses. An excess of heterozygotes and homozygotes are indicated by negative and positive D values, respectively.

The Continuous character, maximum likelihood method was used to construct phylogenetic trees from these data. The Program used CONTML (version 3.4) is part of Felsenstein's (1990) PHYLIP package.

Table 2: Allele frequencies in ocean jackets from three locations. (N = sample size.)

| | Locality | | | |
|---|---|---|---|---|
| Locus | 1 | 2 | 3 | 4 |
| *ADA** | | | | |
| (N) | 87 | 78 | 84 | 25 |
| A | .356 | .244 | .393 | .460 |
| B | .563 | .679 | .512 | .420 |
| C | .063 | .077 | .095 | .120 |
| D | .017 | .000 | .000 | .000 |
| *EST-1** | | | | |
| (N) | 89 | 82 | 95 | 25 |
| A | .045 | .091 | .089 | .460 |
| B | .927 | .890 | .884 | .540 |
| C | .028 | .018 | .026 | .000 |
| *G6PDH** | | | | |
| (N) | 86 | 80 | 95 | 24 |
| A | .047 | .231 | .063 | .250 |
| B | .890 | .725 | .905 | .688 |
| C | .064 | .044 | .032 | .063 |
| *GPI-1** | | | | |
| (N) | 88 | 80 | 95 | 24 |
| A | .028 | .094 | .079 | .354 |
| B | .932 | .869 | .889 | .583 |
| C | .040 | .038 | .032 | .063 |
| *IDH* | | | | |
| (N) | 85 | 81 | 94 | 25 |
| A | .018 | .099 | .106 | .000 |
| B | .982 | .840 | .867 | 1.000 |
| C | .000 | .062 | .027 | .000 |
| *LDH* | | | | |
| (N) | 84 | 82 | 94 | 25 |
| A | .065 | .030 | .005 | .000 |
| B | .935 | .945 | .968 | 1.000 |
| C | .000 | .024 | .027 | .000 |
| *MDH-1** | | | | |
| (N) | 87 | 82 | 95 | 21 |
| A | .029 | .018 | .032 | .095 |
| B | .960 | .982 | .968 | .905 |
| C | .011 | .000 | .000 | .000 |
| *PEP-L** | | | | |
| (N) | 89 | 82 | 95 | 23 |
| A | .028 | .000 | .021 | .152 |
| B | .888 | 1.000 | .905 | .783 |
| C | .051 | .000 | .037 | .065 |
| D | .034 | .000 | .037 | .000 |
| *PGM-1** | | | | |
| (N) | 89 | 82 | 95 | 25 |
| A | .079 | .061 | .011 | .240 |
| B | .882 | .921 | .968 | .680 |
| C | .039 | .018 | .021 | .000 |

Key
1 Port Lincoln
2 Great Australian Bight
3 Coffs Harbour
4 *Scobinichys granulatus*, Tabaco Bay, the Philippines

Table 3: Coefficients for heterozygote deficiency or excess based at 9 loci calculated by chi-square test for Hardy Weinberg expectations. D = genotypic values for heterozygote excess or deficit.

| | Population | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 2 | | | | 3 | | | |
| Locus | Obs heterozygotes | Exp heterozygotes | Fixation index (F) | D | Obs heterozygotes | Exp heterozygotes | Fixation index (F) | D | Obs heterozygotes | Exp heterozygotes | Fixation index (F) | D |
| *ADA-1* | 43 | 47.983 | .104 | -.104 | 32 | 36.897 | .133 | -.133 | 35 | 48.262 | .275 | -.275 |
| *EST-1* | 12 | 12.275 | .022 | -.022 | 14 | 16.299 | .141 | -.141 | 12 | 19.900 | .397 | -.397 |
| *G6PDH* | 13 | 17.413 | .253 | -.253 | 27 | 33.519 | .194 | -.194 | 12 | 16.674 | .280 | -.280 |
| *GPI-1* | 10 | 11.381 | .121 | -.121 | 18 | 18.806 | .043 | -.043 | 17 | 19.153 | .112 | -.112 |
| *IDH-1* | 1 | 2.947 | .661 | -.661 | 18 | 22.815 | .211 | -.211 | 19 | 22.207 | .144 | -.144 |
| *LDH-1* | 5 | 10.280 | .514 | -.514 | 5 | 8.628 | .420 | -.420 | 4 | 5.835 | .314 | -.314 |
| *MDH-1* | 5 | 6.776 | .262 | -.262 | 3 | 2.945 | -.019 | -.019 | 6 | 5.811 | -.033 | .033 |
| *PGM-1* | 11 | 19.073 | .423 | -.423 | 7 | 12.152 | .424 | -.424 | 5 | 5.853 | .146 | -.146 |
| *PEP-L* | 16 | 18.478 | .134 | -.134 | 3 | 2.945 | -.019 | .019 | 16 | 16.847 | .050 | -.050 |

## Results and Discussion

Of the 44 enzymes screened for polymorphism twelve showed no activity. The remaining 32 enzymes represented a total of 38 presumed loci; of these, nine were both polymorphic and could be scored readily. These were *ADA*, EST-1*, G-6PDH*, GPI-1*, IDH*, LDH*, MDH-1*, PEP (leu-tyr)** and *PGM-1**. Two additional loci, *AAT-1** and *ME**, were polymorphic but did not screen reliably so they were omitted from further study (Appendix 3). The nine polymorphic loci were regarded as sufficient for this survey to be extended to compare differences between samples from three localities (see Richardson *et al.*, 1986).

Allele frequencies and the numbers of animals successfully scored for each polymorphic locus at each locality are presented in Table 2. The latter also contains data on the same loci for *Scobinichys granulatus*, collected in Tabaco Bay, the Philippines. This was used in analyses where an outgroup was required. Goodness-of-fit to the Hardy-Weinberg distribution was calculated for each sample and locus (Table 3).

The genotypic D values (coefficient of heterozygote deficit or excess) per locus were calculated and are found in Table 3. These values can be taken as a rough estimate of the joint effects of any external forces that produce a net deviation from Hardy-Weinberg proportions. A negative D value results from a deficit of heterozygotes, a positive value from an excess. Consistent deficits were evident for most loci among fish from the three localities examined.

This widespread heterozygote deficit may be due to:

1) differential selection against heterozygotes
2) mixing of two or more populations of differing allele frequencies at the loci concerned (Wahlund effect)
3) occurrence of uncommon or null alleles (see Andersson *et al.*, 1981).

However we are not able to distinguish between these possibilities on the basis of this study.

Differences between localities

Considering the overall genetic heterogeneity among the three populations, there is evidence of extensive differentiation as revealed by the genic contingency chi-square test for homogeneity (Table 4). Highly significant chi-square values were recorded for all loci (except *MDH**) examined, $p<0.01$ in each case (Table 4).

Table 4: Contingency chi-square analysis based on 9 polymorphic loci.

PL = Port Lincoln, GAB = Great Australian Bight, CH = Coffs Harbour

N = number of alleles, $\chi^2$ = chi-square value, D.F. = degrees of freedom, P = probability

A. For all oceanjacket samples

| Locus | N | $\chi^2$ | D.F. | P |
|---|---|---|---|---|
| *ADA** | 4 | 22.516 | 9 | .00738 |
| *EST-1** | 3 | 73.470 | 6 | .00000 |
| *G6PDH** | 3 | 39.704 | 6 | .00000 |
| *GPI-1** | 3 | 159.190 | 6 | .00000 |
| *IDH** | 3 | 32.165 | 6 | .00002 |
| *LDH** | 3 | 18.270 | 6 | .00559 |
| *MDH-1** | 3 | 6.429 | 6 | .37687 |
| *PGM-1** | 3 | 34.531 | 6 | .00001 |
| *PEP-L** | 4 | 49.453 | 9 | .00000 |
| (Totals) | | 435.728 | 60 | .00000 |

B. PL vs. GAB populations

| Locus | N | $\chi^2$ | D.F. | P |
|---|---|---|---|---|
| *ADA** | 4 | 8.160 | 3 | .04282 |
| *EST-1** | 3 | 3.224 | 2 | .19954 |
| *G6PDH** | 3 | 24.265 | 2 | .00001 |
| *GPI-1** | 3 | 6.392 | 2 | .04092 |
| *IDH** | 3 | 21.886 | 2 | .00002 |
| *LDH** | 3 | 6.216 | 2 | .04470 |
| *MDH-1** | 3 | 2.316 | 2 | .31413 |
| *PGM-1** | 3 | 1.813 | 2 | .40383 |
| *PEP-L** | 4 | 19.571 | 3 | .00021 |
| (Totals) | | 93.843 | 20 | .00000 |

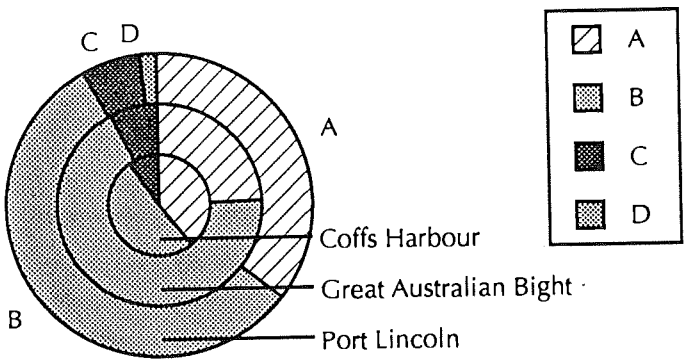
C. PL vs. CH populations

| Locus | N | $\chi^2$ | D.F. | P |
|---|---|---|---|---|
| *ADA** | 4 | 4.730 | 3 | .19269 |
| *EST-1** | 3 | 2.879 | 2 | .23707 |
| *G6PDH** | 3 | 2.493 | 2 | .28758 |
| *GPI-1** | 3 | 4.623 | 2 | .09910 |
| *IDH** | 3 | 16.751 | 2 | .00023 |
| *LDH** | 3 | 14.098 | 2 | .00087 |
| *MDH-1** | 3 | 2.215 | 2 | .33034 |
| *PGM-1** | 3 | 11.577 | 2 | .00306 |
| *PEP-L** | 4 | .641 | 3 | .88690 |
| (Totals) | | 60.007 | 20 | .00000 |

D. GAB vs. CH populations

| Locus | N | $\chi^2$ | D.F. | P |
|---|---|---|---|---|
| *ADA** | 3 | 9.762 | 2 | .00759 |
| *EST-1** | 3 | .258 | 2 | .87889 |
| *G6PDH** | 3 | 21.306 | 2 | .00002 |
| *GPI-1** | 3 | .353 | 2 | .83810 |
| *IDH** | 3 | 2.632 | 2 | .26816 |
| *LDH** | 3 | 3.320 | 2 | .19013 |
| *MDH-1** | 2 | .627 | 1 | .42842 |
| *PGM-1** | 3 | 6.854 | 2 | .03248 |
| *PEP-L** | 4 | 16.369 | 3 | .00095 |
| (Totals) | | 61.481 | 20 | .00000 |

a) ADA




b) G6PDH

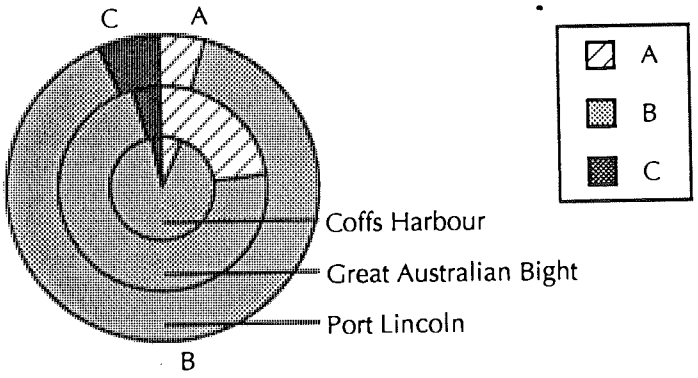



Figure 2: Pie graphs of allele frequencies at the a) *ADA** and b) *G6PDH** loci in each population studied.

Table 5: Summary of F-statistics based at 9 polymorphic loci for all ocean jacket samples.

| Locus | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| *ADA** | .271 | .291 | .027 |
| *EST-1** | .241 | .369 | .169 |
| *G6PDH** | .483 | .505 | .044 |
| *GPI-1** | .286 | .534 | .348 |
| *IDH** | .211 | .253 | .053 |
| *LDH** | .437 | .448 | .020 |
| *MDH-1** | .101 | .110 | .009 |
| *PGM-1** | .600 | .626 | .066 |
| *PEP-L** | .256 | .289 | .045 |
| Mean | .321 | .380 | .087 |

Table 6: Different matrices of genetic similarity and/or distance coefficients based at 9 loci in four populations (including an outgroup population)

A. Below diagonal: Nei's (1972) genetic distance*
Above diagonal: Nei's (1972) genetic identity*

| | Population | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | PORTLINC, SA | | .992 | .997 | .924 |
| 2 | GBIGHT, SA | .008 | | .993 | .928 |
| 3 | COFFS HARBOUR, NSW | .003 | .007 | | .930 |
| 4 | TABACO BAY, PHIL | .079 | .075 | .072 | |

B. Below diagonal: CSE (1967) chord distance*
Above diagonal: Nei's (1972) minimum distance

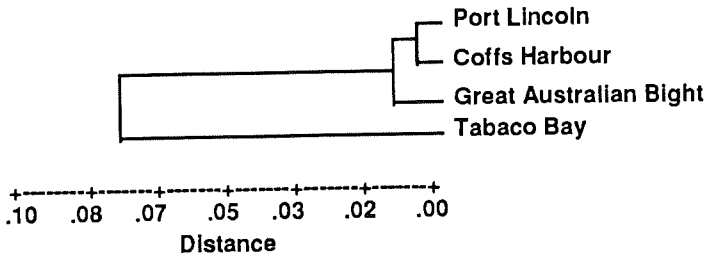
| | Population | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | PORTLINC, SA | | .007 | .003 | .062 |
| 2 | GBIGHT, SA | .124 | | .006 | .057 |
| 3 | COFFS HARBOUR, NSW | .101 | .100 | | .057 |
| 4 | TABACO BAY, PHIL | .234 | .223 | .229 | |

C. Below diagonal: Mod. Rogers distance (Wright, 1978)*
Above diagonal: Nei's (1972) genetic distance

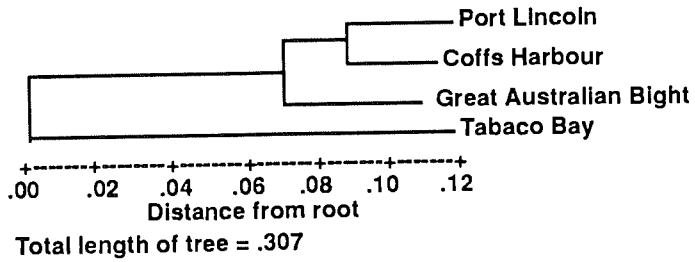
| | Population | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | PORTLINC, SA | | .008 | .003 | .079 |
| 2 | GBIGHT, SA | .083 | | .007 | .075 |
| 3 | COFFS HARBOUR, NSW | .051 | .078 | | .072 |
| 4 | TABACO BAY, PHIL | .248 | .239 | .238 | |

* indicates a constructed dendrogram
CSE = Cavalli-Sforza and Edwards.

A. Nei's (1972) genetic distance




B. Modified Rogers distance - OPT (Wright, 1978)




C. Cavalli-Sforza & Edwards (1967) chord distance (OPT)

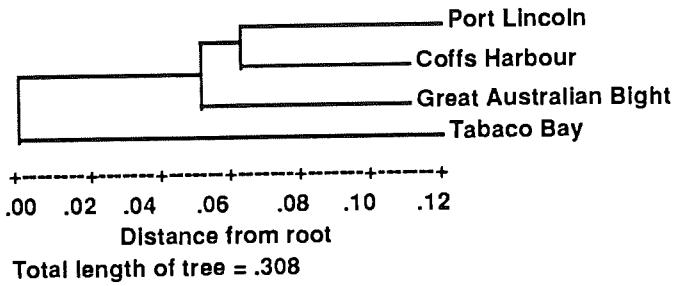



Figure 3: Dendrograms constructed employing different measures of genetic similarity and/or distance.
A uses UPGMA clustering;
B and C use Distance Wagner clustering

Due to their different combination of loci in each population and with only three populations combined, the results are probably random statistical departures. The pairwise comparisons between populations exhibit significant differences at three loci (Table 4). The three loci concerned vary between comparison pairs. The differences between two of these, *ADA** and *G-6PDH**, at the different localities are illustrated as pie diagrams in Figure 2.

The F-statistic (Wright, 1978) is a measure of genetic differentiation between populations. The $F_{IT}$ and $F_{IS}$ refer to the fixation indices relative to the total population and its subpopulations, respectively, while $F_{ST}$ measures the level of differentiation among subpopulation relative to the amount under complete fixation. The $F_{IT}$ values for each locus, representing the deviation from Hardy-Weinberg proportions of the pooled samples, were all positive, indicating an overall deficit of heterozygotes, which in turn reflects inter-population heterogeneity. All observed $F_{IS}$ values were positive which indicates further subdivision within a sample. The $F_{ST}$ values ranged from 0.009 (*MDH-1**) to 0.348 (*GPI-1**) with a mean value of 0.087 (Table 5). These values indicate low to moderate differentiation among the populations surveyed.

The genetic differentiation between populations was further investigated by calculation and comparison of mean coefficients of similarity indices and distance coefficients. The results of comparisons made using Nei's (1972) genetic distance. Nei's (1978) unbiased genetic identity, the modified Roger's distance (Wright, 1978) and Chord distance (Cavalli-Sforza and Edwards, 1967) are found in Table 6.

Dendrograms were constructed to show the genetic relationships between the samples. Some of these are shown in Figure 3. The dendrograms include *S. granulatus* from Tabaco Bay, the Philippines, which was used as an outgroup. In all cases the relationships between the fish sampled form the different localities were the same. Those from Port Lincoln (S.A.) and Coffs Harbour (N.S.W.) were the first to join the cluster followed by Great Australian Bight. This indicates that the fish from Port Lincoln and Coffs Harbour were more closely related to each other than either were to those from the Great Australian Bight. This is surprising given the geographic locations of the sampling sites.

Morphometric and meristic characteristics are often used in attempts to distinguish between sub-populations of fish (Ihssen *et al.*, 1981). Such studies were undertaken on ocean jackets by Musa (1994) and the appropriate chapter of her thesis is found as Appendix 4 of this report. She used the same fish as were

used in the genetic studies. The main finding of this study was that there was considerable overlap between the samples. However the Port Lincoln fish were more closely related to those from Coffs Harbour than to those from the geographically closer Great Australian Bight. This is the same result as found with the genetic analysis.

The major fishery for this species is in South Australia; at this stage catches in other states are very small. The possibility of population subdivision in South Australian waters is an important issue which should be addressed as soon as possible to provide information to the managers of the fishery.

This pilot study revealed sufficient genetic variability for isozyme analysis to be a useful means of studying population sub-structuring in ocean jackets. However the study itself was of small scale and did not address to any extent temporal variation within each site. Further detailed studies over a 2-3 year period are required before any firm conclusions about stock structure are made. These should follow the recommendations of Richardson *et al.* (1986) and include samples from a wider geographic range. Additional studies could also include other biochemical methods eg mtDNA analyses, both restriction fragment analysis and sequencing. These additional methods would result in broader understanding of the genetic structure of ocean jacket populations.

As well as understanding the genetic structure of the populations more biological information is needed. For example, age and growth rate at different locations, migration distances etc are needed before appropriate biologically based management decisions can be made.

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## Appendices

Appendix 1: Enzymes investigated in N. ayraudi.

| Enzyme | Abbreviation | Enzyme Commission Number |
|---|---|---|
| Aspartate aminotransferase | AAT* | EC 2.6.1.1 |
| Aconitate hydratase | AH* | EC 4.2.1.3 |
| Acid phosphatase | ACP* | EC 3.1.3.2 |
| Adenosine deaminase | ADA* | EC 3.5.4.4 |
| Alcohol dehydrogenase | ADH* | EC 1.1.1.1 |
| Aldehyde dehydrogenase | ALDH* | EC 1.2.1.3 |
| Alkaline phosphatase | ALP* | EC 3.1.3.1 |
| Adenylate kinase | AK* | EC 2.7.4.3 |
| Aldehyde oxidase | AO* | EC 1.2.3.1 |
| Creatine kinase | CK* | EC 2.7.3.2 |
| Diaphorase | DIA* | EC 1.6.2.2 |
| Enolase | ENO* | EC 4.2.1.11 |
| Esterase | EST* | EC 3.1.1.1 |
| Fructose diphosphatase | FDP* | EC 3.1.3.11 |
| Fumarate hydratase | FH* | EC 4.2.1.2 |
| Galactose dehydrogenase | GALDH* | EC 1.1.1.48 |
| Glyceraldehyde-3-phosphate-dehydrogenase | GAPDH* | EC 1.2.1.12 |
| Guanine deaminase | GDA* | EC 3.5.4.3 |
| Glucose dehydrogenase | GDH* | EC 1.1.1.47 |
| Glucose-6-phosphate dehydrogenase | G6PDH* | EC 1.1.1.49 |
| Alpha-glycerophosphate dehydrogenase | GPD* | EC 1.1.1.8 |
| Glucose-6-phosphate isomerase | GPI* | EC 5.3.1.9 |
| Glutamate-pyruvate transaminase | GPT* | EC 2.6.1.2 |
| Glycerate dehydrogenase | GLYDH | EC 1.1.1.29 |
| Glutamate-oxaloacetate transaminase | GOT/AAT*. | EC 2.6.1.1 |
| Glycollate oxidase | GOX* | EC 1.1.3.1 |
| Hexokinase | HK* | EC 2.7.1.1 |
| Hexosaminidase | HEX* | EC 3.2.1.52 |
| Isocytrate 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 | sMDH* | EC 1.1.1.37 |
| Malate dehydrogenase | mMDH* | EC 1.1.1.37 |
| Malic enxyme | ME* | EC 1.1.1.38 |
| Mannose-6-phosphate isomerase | MPI* | EC 5.3.1.8 |
| Nucleoside phosphorylase | NP* | EC 2.4.2.1 |
| Peptidase A | PEP A* | EC 3.4.1.1 |
| Peptidase B | PEP B* | EC 3.4.1.1 |
| Peptidase (leu-tyr) | PEP(leu-tyr)* | EC 3.4.1.1 |
| Peptidase (leu-leu-leu) | PEP* | EC 3.4.1.1 |
| Phosphogluconate dehydrogenase | PGDH* | EC 1.1.1.44 |
| Phosphoglucomutase | PGM* | EC 5.4.2.2 |
| Pyruvate kinase | PK* | EC 2.7.1.4 |
| Sorbitol dehydrogenase | SDH* | EC 1.1.1.14 |
| Xanthine dehydrogenase | XDH* | EC 1.1.1.204 |

## Appendix 2: Enzymes, buffers and tissues screened in *N. ayraudi.*

Key: L = liver, M = muscle, H = heart.
* = best tissue/buffer for this enzyme.
1 = Tris -EDTA-boric acid (TBE) pH 9.0
2 = Tris-citric acid (TC) pH 6.8
3 = Tris citric acid (TC) pH 5.8
4 = Tris-maleate (TM) pH 7.8
5 = Citric acid-aminopropyl-morphine (CAM) pH 6.1
6 = Poulik
A = Anodal
P = Polymorphic

| Enzyme | Tissue | Buffers | Presumed # of Loci | Comments |
|---|---|---|---|---|
| AAT | L* | 1 2* 3 4 5 6 | 2A | good activity and resolution, AAT-1* P |
| | M | 1 2 3 4 5 6 | | fair activity and resolution |
| | H | 1 2 3 4 5 6 | | poor activity and resolution |
| AH | L* | 1 2* 3 4 5 6 | 1A | poor activity and resolution |
| | M | 1 2 3 4 5 6 | | no staining |
| | H | 1 2 3 4 5 6 | | no staining |
| ACP | L* | 1 2* 4 | 2A | poor activity and resolution |
| | M | 1 2 4 | | no staining |
| | H | 1 2 4 | | no staining |
| ADA | L* | 1 2 3* 4 5 6 | 1A | good activity and resolution, P |
| | M | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| ADH | L | 1 2 3 4 | 1A | fair activity and resolution, scorable |
| | M | 1 2 3 4 | 1A | fair activity and resolution |
| | H | 1 2 3 4 | | fair activity and resolution |
| ALDH | L | 1 2 3 4 | | no staining |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| ALP | L | 1 2 3 4 | | no staining |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| AK | L | 1* 2 3 | 1A | good activity and resolution |
| | H | 1 2 3 | 1A | fair activity and resolution |
| AO | L | 1 2 3 4 | 1A | fair activity and resolution |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| CK | L* | 1 2 3* 4 | 1A | fair activity and resolution |
| | M* | 1 2* 3 | 1A | good activity and resolution, P? |
| | H | 1 2 3 | 1A | fair activity and resolution |
| DIA | L | 1 2 3 4 | | no staining |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| ENO | L | 1 2 3 4 | | no staining |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| EST | L* | 1* 2 3* 4 5 6 | 2A-variable | good activity and resolution, EST-1* P |
| | M | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| FDP | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| FH | L* | 1* 3 4 | 1A | fair staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |

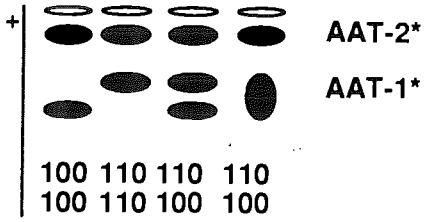
| Enzyme | Tissue | Buffers | Presumed | Comments |
|---|---|---|---|---|
| | | | # of Loci | |
| GALDH | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| GAPDH | L* | 1* 3 4 | 1A | fair activity and resolution |
| | M* | 1 3* 4 | 1A | good activity and resolution |
| | H | 1 3 4 | 1A | fair activity and resolution |
| GDA | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| GDH | L* | 1* 2 3 4 | 1A | fair activity and resolution |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| G6PDH | L* | 1* 2 3* 4* 5 6 | 1A-variable | good activity and resolution, P |
| | M | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| GPD | L | 1 2 3 4 | 1A | poor activity and resolution |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| GPI | L* | 1* 2 3 4 5 6* | 2A-variable | good activity and resolution, P |
| | M* | 1 2 3 4* 5 6 | 2A | good activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| GPT | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| GLYDH | L | 1 3 4 | 1A | poor activity and resolution |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| GOT/AAT | L | 1 3 4 | 2A | fair activity and resolution |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| GOX | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| HK | L | 1 3 4 | 1A | poor activity and resolution |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| HEX | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| IDH | L* | 1 2 3* 4 5 6 | 1A | good activity and resolution, P |
| | M | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| LDH | L* | 1* 2 3 4 5 6 | 1A | good activity and resolution, P |
| | M* | 1 2 3 4* 5 6 | 1A | good activity and resolution, P |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| LAP | L | 1 3 4 | | no staining |
| | M | 1 3 4 | | no staining |
| | H | 1 3 4 | | no staining |
| MDH | L* | 1* 2 3 4 5 6 | 2A-variable | good activity and resolution, MDH-1* P |
| | M* | 1 2 3 4* 5 6 | 1A | good activity and resolution, P |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| ME | L* | 1 2 3 4* 5 6 | 1A | good activity and resolution, ME-1* P |
| | M* | 1 2 3* 4 5 6 | 2A | good activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |
| MPI | L* | 1 2 3 4* 5 6 | 1A | fair activity and resolution, scorable, P ? |
| | M* | 1 2 3 4 5* 6 | 1A | fair activity and resolution |
| | H | 1 2 3 4 5 6 | 1A | fair activity and resolution |

| Enzyme | Tissue | Buffers | Presumed # of Loci | Comments |
|---|---|---|---|---|
| NP | L | 1 2 3 | | no staining |
| | M | 1 2 3 | | no staining |
| | H | 1 2 3 | | no staining |
| PEP | L* | 1 3 4* 5 6 | 2A | good activity and resolution |
| (lgg) | M | 1 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 3 4 5 6 | 1A | fair activity and resolution |
| PEP | L* | 1 3 4* 5 6 | 2A-variable | good activity and resolution |
| (leu-pro) | M | 1 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 3 4 5 6 | 1A | fair activity and resolution |
| PEP | L* | 1* 3 4 5 6 | 1A | good activity and resolution, P |
| (leu-tyr) | M | 1 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 3 4 5 6 | 1A | fair activity and resolution |
| PEP | L* | 1* 3 4 5 6 | 2A-variable | fair activity and resolution, P |
| (leu-leu-leu) | M | 1 3 4 5 6 | 1A | fair activity and resolution |
| | H | 1 3 4 5 6 | 1A | fair activity and resolution |
| PGDH | L* | 1 2* 3 4 | 1A | fair activity and resolution, scorable |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| PGM | L* | 1* 3 4 5 6 | 2A-variable | good activity and resolution, P |
| | M* | 1 3 4 * 5 6 | 1A | good activity and resolution, P |
| | H | 1 3 4 5 6 | 1A | fair activity and resolution |
| PK | L | 1 2 3 4 | | no staining |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| SDH | L* | 1 2* 3 4 | 1A | poor activity and resolution |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |
| XDH | L | 1 2 3 4 | | no staining |
| | M | 1 2 3 4 | | no staining |
| | H | 1 2 3 4 | | no staining |

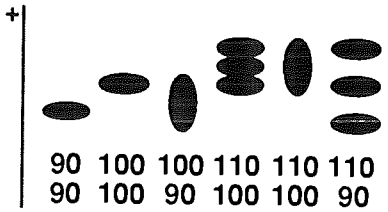
Appendix 3: Observed banding patterns at 11 polymorphic loci in *N. ayraudi*

**Key:** **dark bands** **light bands** **artefact or/and subbands**

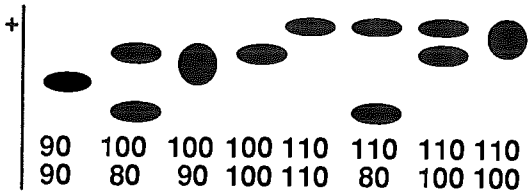
*AAT*



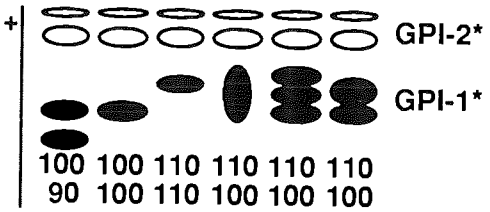

*G6PDH*



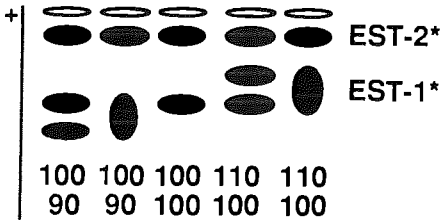

*ADA*



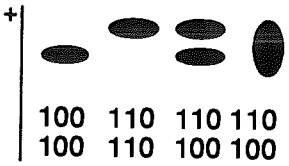

*GPI*



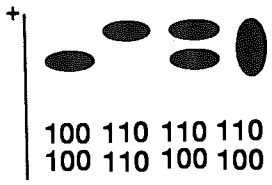

*EST*



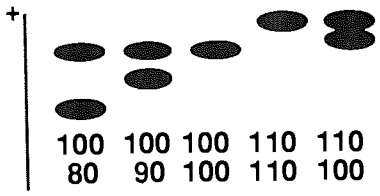

*IDH*



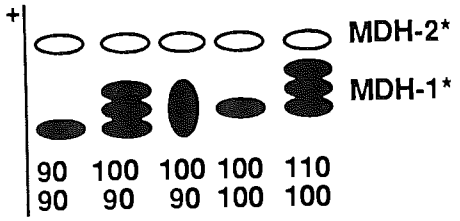
*LDH*



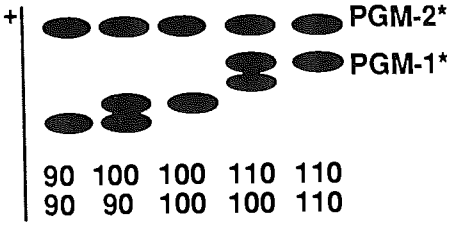

*PEP leu-tyr*



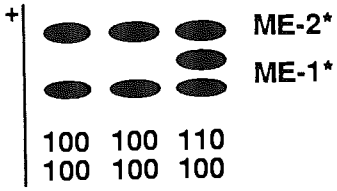

*MDH*




*PGM*




*ME*



Appendix 4

# MORPHOMETRIC AND MERISTIC STUDY

(Chapter 4 in Musa, J. C., 1994, Genetic Variation in Seven Species of Leatherjackets (Pisces: Monacanthidae) with special reference to the Population Structure of Ocean jackets (*Nelusetta ayraudi*), unpublished MSc thesis, University of New South Wales.)

## 1. Introduction

The collection of morphometric data from the specimens examined by electrophoresis was necessary for the correct identification of species, an essential aspect of any taxonomic study. It also provide an independent data set for elucidation of species relationships and to classify and identify populations and/or stocks. Phenotypic expression of any body form and shape is known to involved both genetic and environmental influence, although the correlation of morphology and genetics is not well understood at this stage. The degree of morphological variation that is influenced by environmental variability is difficult to measure because some environmental factors could not be absolutely controlled. Kirpichnikov (1987) provides a thorough summary of the existing literature concerning genetic and morphological variability arising from fish breeding studies.

Many investigators have used meristic and morphometric characters to delineate races, populations and/or stocks of fishes (Merriman and Thorpe 1976, Lear and Sandeman 1980, MacCrimmon and Claytor 1984, O'Maoileidigh *et al.* 1988, Leslie and Grant 1990 and Melvin *et al.* 1992. The practice of segregating groups, especially fishes, based on differences in vertebral counts, lateral line scale counts, fin ray counts and morphometric distances is well entrenched in systematics (Sneath and Sokal 1973) and fisheries biology (Royce 1964). Some authors have even described separate species solely on the basis of differences in a single character (Brown 1965).

The species under investigation, ocean jackets, are taken as a by-catch in commercial trawls and the annual catch is small relative to that of other commercial marine fishes, typically up to 900 tons (Grove-Jones 1990).

As ocean jackets constitute the highest marine scale fishery by weight in SA, they constitute an important part of the harvest (Grove-Jones and Burnell 1991) and

there is an increasing interest by commercial fishermen in locating and exploiting areas of ocean jacket abundance. There is, however, no stated management policy because the ocean jacket harvest is solely incidental and the catch is difficult to estimate because they are usually sold to local markets (Dixon pers. comm.).

In this study, morphometric and meristic characters were used to examine populations of ocean jackets for evidence of geographic stock structure and results were used to complement the genetic study discussed in chapter 3. This is necessary to know if ocean jacket in Australian waters belong to one interbreeding populations or if a separate subpopulation exists. If an evidence of a reproductively isolated stocks occur among the three populations surveyed, morphological differences between stocks may be apparent. Hence, if differences is found, individual or regional stocks could be identified which could be used as a management tool for this resource. There are no studies yet undertaken with regards to a detailed morphological examination on this species hence, results could add to the limited knowledge of the ocean jacket biology. Finally, this chapter compares morphometric and meristic characters of ocean jackets in three geographic areas, Port Lincoln and Great Australian Bight (SA) and Coffs Harbour in New South Wales.

## 2. Methods:

### 2.1 Sample collection

Random samples chosen from three collection sites of ocean jackets used for the electrophoresis as described in Chapter 2 were used in the morphometric analysis.

### 2.2 Morphometric characters

The same morphometric measurements employed by Laevastu (1965), Dixon *et al.* (1987) and Syahailatua (1992) were used in the present study. Table 4.0 present the 15 morphometric characters measured in this study. Figure 4.0 showed the diagram of the morphological features of ocean jacket. Total, caudal and standard length were measured with a metal rule to the nearest 1.0 mm and all other measurements were made with slide digital callipers (Max-cal NSK) to the nearest 0.01 mm. All measurements were taken from thawed specimens.

2.3 Meristic characters

Meristic data consisted of the numbers of dorsal and anal fin rays, number of gill rakers and number of vertebrae. Accurate fin rays counts were ensured by using binocular microscope. Similar technique was used for gill raker counts. Vertebral counts were made by dissection.

## 3. Statistical Analysis

In the initial analysis, variation in the mean size of the fish among samples dominated the results. The mean values for each character followed the same pattern as those for SL, i.e. area 3> area 2> area 1. The Duncan's multiple range test and T-tests for each character reflected a similar pattern; no significant difference between areas 1 and 2 or between areas 2 and 3. The difference in SL among areas may not be a true reflection of population differences, but it may result from sampling bias. Furthermore, the dominance of the size component in the analysis may have masked more subtle differences in "shape" among areas.

Normality of data is a basic assumption of many multivariate statistical analyses (i.e. stepwise discriminant; principal component, etc). Therefore, each variable of the three groups was checked for outliers both before and after data transformation. The jacknife method and Box-Cox family of transformations were used for deleting outliers and data transformation, respectively. All morphometric characters were transformed to log 10 values for all statistical analyses (Appendix 7, Musa, 1994). Analysis of variance with standard length as the covariate was used to standardise the morphometric characters to a common size (Gould 1966, Thorpe, 1976, Ihssen *et al.* 1981, MacCrimmon *et al.* 1983, Beacham, 1984 and Reist 1985). Meristic data were transformed into square roots (Sokal and Rohlf, 1981) (Appendix 8, Musa, 1994). All statistical analyses were made with the SAS (Statistical Analysis Systems, 1989) computer program.

Significant differences for the meristic and the adjusted morphometric characters were determined using the analysis of variance and analysis of covariance procedures of the SAS computer program. T-tests and Duncan's multiple range tests for each character was calculated to determined the differences or similarity among populations. Univariate and multivariate comparisons were made on meristic and morphometric data separately.

Multivariate classification methods were used to discriminate among the three areas. Principal component analysis was employed to examine the overall pattern of morphological covariation. The factor loadings produced by PCA were

used to estimate the scores of the first three principal components of each individual for graphical examination. Although PCA does not take any *a priori* group structure in the total sample into account, it nevertheless give an overview of the groupings of individuals in Euclidean space (Somers 1986). When there is information about group structure, canonical variate analysis (CVA) or multigroup PCA may be used. However, one drawback with CVA is that factors are not as easily interpreted as principal components (Sundberg 1988).

Subsets of meristic and morphometric characters that best discriminated among the three areas were selected by stepwise discriminant function analysis (SDA). The selected subsets of characters were employed to generate a discriminant function (DF) for classifying individuals to area. The use of this characters alone increases the Euclidean distances (Somers 1986) between groups. Another advantage of the SDA is that fish with full data sets for the selected characters can be included in the DF analysis despite having missing values for unselected characters (Leslie and Grant 1990).

One assumption of the linear DF is that within-group covariance matrices are not significantly different among groups. Tests for homogeneity (Morrison 1976), however, detected significant differences between the within-sample covariation for the morphometric data ($X^2$=64.3, df=18, $p< 0.0001$), but not for meristic data. Leslie and Grant (1990) noted that when there is significant heterogeneity, the quadratic form of the DF should be used. However, Wahl and Kronmal (1977) have shown that, even when the assumption of homogeneous within-sample covariation is violated, the linear DF outperforms the quadratic DF when sample sizes are small (fewer than 25 individuals per sample with four characters). As sample sizes in this study were within the range of this minimal value, the linear DF was applied in the analysis of both morphometric and meristic data. The discriminant functions were used to assigned individuals to areas with prior probabilities of classification set equal among the areas and not proportional to sample size. The test of the discriminant function was, therefore considered to be more rigorous.

## 4. Results

### 4.1 Morphometric characters

Means, coefficient of variation and sample sizes are presented in Table 4.1. The analysis of variance (ANOVA) revealed a significant overall degree of heterogeneity in the morphometric measurements among areas ($p<0.01$). The

ANOVAs demonstrated significant differences among areas for SL, HW, SNL, VED, MBH and SPF. Multiple range comparisons of characters among areas are presented in Table 4.2a and 4.2b. The means of the size-free values of these six characters showed a regular geographic cline SL, SNL, MBH and SPF increased from area 3 (CH) to area 2 (GAB ) and then to area 1 (PL), whereas the other two characters (HW and VED) increased from area 3 to area 1 and to area 2. For SL, SNL and MBH, area 1 was significantly different from area 3, but was not significantly different from area 2. For HW, areas 3 and 1, and areas 3 and 2 were significantly different from one another, but areas1 and 2 were not significantly different from each other. For VED, area 3 and area 2 were significantly different from each other, but all three areas were not significantly different from one another.

The PC1 represented 93.09% of the variation after size had been extracted. Thirteen of the fifteen morphometric characters measured showed large positive loadings except the lengths of the dorsal and pectoral fins with negative loadings (Table 4.3). Fish with long dorsal and pectoral fins relative to the overall size tended to have lower scores on PC1. CFL, SL, SDF, and SAF had the highest covariation with PC1. MBH showed negative loading on PC2 and all other loadings were positive which accounted for 97% of the variation. PC3 represented 48.57% of the variation and mainly reflected variation in SDF and SAF, which is negatively correlated with this component. Figure 4.1 showed the lower scores on PC1 for fish with greater body height relative to the overall body size. The summary of the PC analysis is shown in Appendix 9 (Musa, 1994).

The stepwise discriminant function analysis selected six morphometric variables (HW, VED, SNL, AFL, CFL and SL), in order of importance, as the characters that best discriminated among areas (Table 4.4). The discriminant function performed better, although comparable with that of the function based on meristic counts having only four characters measured. Fish from area 1 and 2 had the same values of correct classification (95%) whereas fish from area 3 had 85%. Area 1 has the greatest percentage of misclassification (38.33%) than the other two areas. Fish from areas 1 and 2 were most distinct from area 3 although tended to be classified equally as shown in the slight differences of their classification values (Table 4.5). The summary of the SDF analysis for all characters is presented in Appendix 10 (Musa, 1994).

The Mahalanobis distances between areas presented in Table 4.6 of morphometric characters ranged from 19.67 to 4.48 and were greater between

more distantly located areas. The result of the morphometric study suggested a possible differentiation along the SA and NSW samples.

4.2 Meristic characters

The means, coefficient of variation and sample sizes are shown in Table 4.1. The ANOVA detected a non significant overall degree of heterogeneity in the meristic characters among areas. There was however, significant difference among areas for only one meristic character, NGR

(F=2.61, p=0.081), out of four characters measured. Multiple range comparisons of means showed that fish from Area1 had significantly fewer NGR than fish from other areas (Table 4.2b). Fish from area 2 had significantly fewer NGR than fish from area 3, but were not significantly different from each other.

The principal component analysis of the meristic characters is presented in Table 4.3. The first two eigenvalues of the PCA were greater than unity and together explained 99.53% of the total variation. The addition of the third eigenvalue (0.621) accounted for a very minimal addition to the total variation, so that the first three principal components (PC1 - PC3) accounted for 100 percent of the total variation.

The elements of PC1 were all positive except for the number of vertebrae (NV) which has a very small negative loading and was not correlated with the vector. This vector was interpreted as a magnitude vector on which fish with higher meristic counts loaded heavily. Number of dorsal fin (NDF) and number of anal fin (NAF) were positively correlated with this vector ($r^2$ = 0.101 and 0.114 respectively). Loadings on PCA contrasted fin ray count (NDF), negative loading and number of gill rakers (NGR), with the last count, number of vertebrae (NV), negative loading (Table 4.3).

There was a relatively large degree of overlap in the scores of individuals from different areas on PC1 and PC2 and no clear group structure was apparent (Figure 4.2). Analysis of variance of the individual PCA loadings revealed significant difference only in one meristic character, NGR, (F=2.61, p=0.081) among areas. The PCA reflected the results of the univariate analyses of individual meristic counts. From these results, fish from area 1 would be expected to have lower scores for both PC1 and PC2.

The stepwise discriminant analysis selected a single meristic character (NGR), as the character that best discriminated among areas. The linear equation

generated by the SDF analysis of this character did not discriminate well among fish from different areas. Fish from area 1 (PL) and area 2 (GAB) had the greatest proportions of correct classifications (Table 4.5) and fish from area 3 (CH) was misclassified into these areas. No classification was given for area 3 based on meristic characters and was expected because of the zero values calculated during the pairwise generalised squared distance between areas. Hence, fish from this area was misclassified into area 1 and area 2.

The Mahalanobis distances D2 between areas ranged between 0.227 and 0.486 (Table 4.6). The largest distance was between Areas 1 and 3 and reflected the differences detected in the foregoing analyses. The values of the Mahalanobis distances appeared to reflect geographic distance between area 1 and 2 than area 2 and 3.

**4. Discussion**

Stocks of marine fish can be defined in two ways. First, they are "a group of fish exploited in a specific area or by a specific fishing method" (Smith and Jamieson 1986). In this definition, stock boundaries may be set as a matter of convenience to fishery managers and may not necessarily have any biological significance. Stocks limit can be determined by such criteria as territorial boundaries, lines of latitude or longitude on shore. Second, fish stocks are considered as biological entities, in which group of fish are partially or completely reproductively isolated from other groups (Leslie and Grant 1990). These stocks are usually defined in terms of the genetic concept of a population, a collection of randomly mating individuals (Ihssen *et al.*1981). The geographic limits of such group may be set by such barriers to adult migration or larval drift as temperature and salinity discontinuities and diverging current systems.

The ANOVAs of the morphometric and meristic variation revealed a small amount of differentiation among the three areas. Adjoining samples were not significantly different from each other for most of the characters, but widely separated samples did show some significant differences. Likewise, Mahalanobis distances, which are an overall measure of morphological differentiation, were smallest between adjoining samples. The results suggests that ocean jackets may be sufficiently confined within an specified area after settlement to develop small morphological differences among areas.

Morphological differences can accumulate in different environments. Hubbs (1926) demonstrated that numbers of vertebrae and fin rays are influenced

by water temperature during larval development, higher temperature leading to lower counts.

There was a gradual decrease in the average number of gill rakers from area 1 in NSW to area 3 in SA. Mean count for the number of gill rakers was not significantly different among areas and was attributable to the small number of individual samples for each of the area studied. An increase in the number of individuals per sample area may result in the same level of significant difference in between all areas.

The over-riding result of the morphometric analyses before size was extracted was that fish were largest in Coffs Harbour (Area 3) and smaller in Port Lincoln (Area 1) and Great Australian Bight (Area 2). The size difference may be due to sampling bias. The SA samples were collected by commercial trawlers and smaller fish may have been selected for scientific sample. The average size of these fish, however, were not significantly smaller than that of fish collected randomly from Coffs Harbour. Alternatively, the differences in size among samples may reflect geographic differences in fishing intensity or growth rates. South Australian region has a greater diversity of habitats and a greater number of species and covering a wide range of geographic areas than the NSW Coffs Harbour and may therefore provide better foraging opportunities for ocean jackets (Fricke 1980 and Nelson 1984)

The lack of significant differences between areas suggest that a common origin of fish for all areas may be apparent. However, further work is necessary before any firm conclusion could be made. The comparison of other several methods e.g. truss versus conventional and the use of more elaborate and accurate electronic calipers will possibly lead to concrete database of ocean jacket morphology. In addition, the examination of large sample sizes from different geographic stocks is highly recommended. Finally, the avenues of morphological and biological research in the Monacanthidae family is very promising. The large number of morphological characters that best discriminated the areas surveyed would lead to pursuance of immediate studies in ocean jackets morphology.

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Table 4.0 Measurements of ocean jackets used in this study.

| Abbreviation | Definition |
|---|---|
| | Morphometric |
| CFL | Caudal fork length |
| SL | Standard length |
| SDF | Tip of snout to insertion of dorsal fin |
| SAF | Tip of snout to insertion of anal fin |
| DFL | Dorsal fin length |
| AFL | Anal fin length |
| CPW | Caudal peduncle width |
| HL | Head length |
| HW | Head width |
| SNL | Snout length |
| HED | Horizontal eye diameter |
| VED | Vertical eye diameter |
| MBH | Maximum body height |
| SPF | Tip of snout to insertion of pectoral fin |
| PL | Pectoral fin length |
| | Meristic |
| NDF | Number of dorsal fin rays |
| NAF | Number of anal fin rays |
| NGR | Number of gill rakers |
| NV | Number of vertebrae |

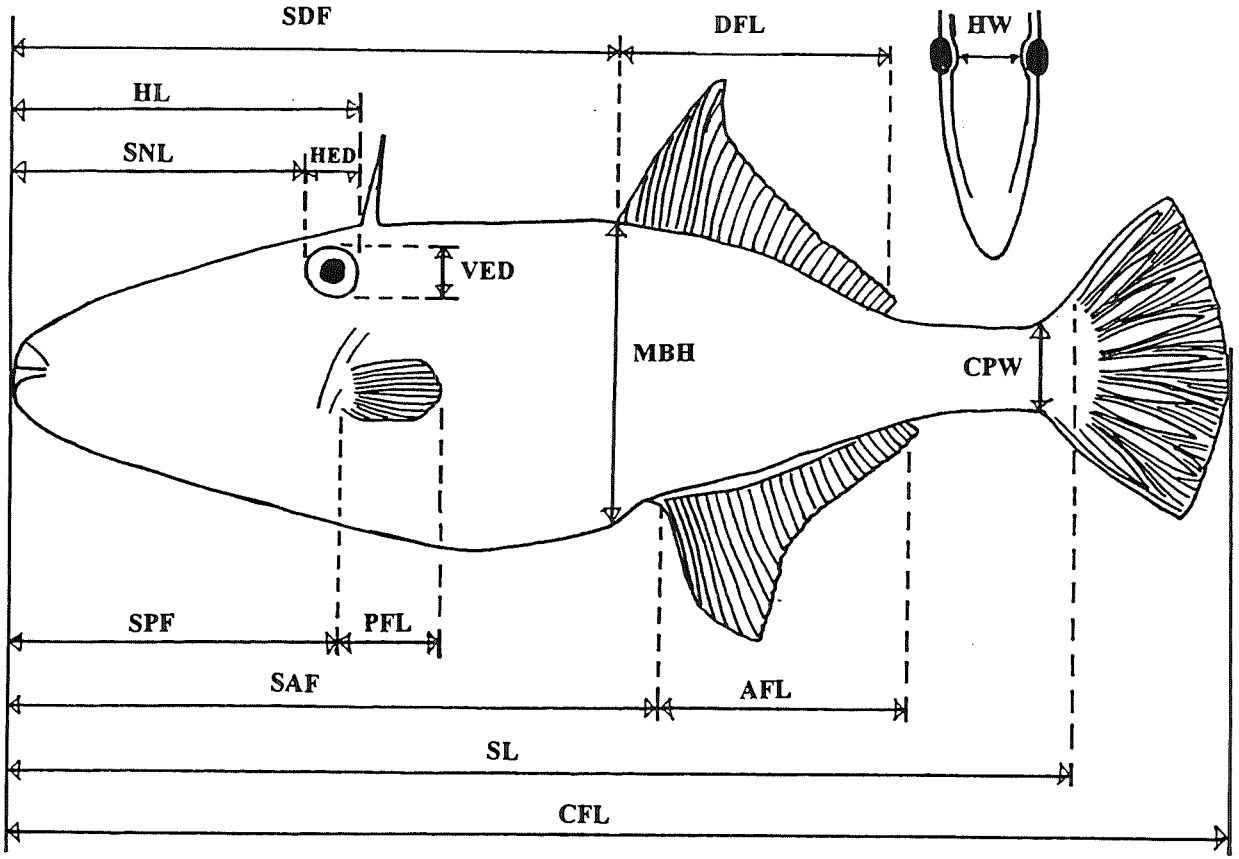


**Figure 4.0 The diagram of morphometric measurements (Descriptions in Table 4.0).**

Table 4.1 Summary of log transformed morphometric and meristic characters of all populations.

| Character | Mean | CV | n |
|---|---|---|---|
| **Morphometric** | | | |
| Caudal fork length | 390.17 | 12.44 | 58 |
| Standard length | 335.85 | 13.06 | 58 |
| Tip of snout to insertion of dorsal fin | 189.45 | 13.03 | 58 |
| Tip of snout to insertion of anal fin | 201.73 | 13.15 | 58 |
| Dorsal fin length | 93.09 | 14.70 | 58 |
| Anal fin length | 0.01 | 29.46 | 58 |
| Caudal peduncle width | 0.04 | 12.41 | 58 |
| Head length | 119.37 | 12.61 | 58 |
| Head width | 25.74 | 11.01 | 58 |
| Snout length | 93.54 | 13.40 | 58 |
| Horizontal eye diameter | 19.18 | 8.17 | 58 |
| Vertical eye diameter | 16.90 | 8.15 | 58 |
| Maximum body height | 103.25 | 15.39 | 58 |
| Tip of snout to insertion of pectoral fin | 103.73 | 13.61 | 58 |
| Pectoral fin length | 36.26 | 10.01 | 58 |
| **Meristic** | | | |
| Number of dorsal fin rays | 5.71 | 1.06 | 58 |
| Number of anal fin rays | 5.59 | 1.22 | 58 |
| Number of gill rakers | 5.52 | 1.89 | 58 |
| Number of vertebrae | 4.24 | 0.36 | 58 |

CV = coefficient of variation
n = sample size

Table 4.2a: Multiple range tests of morphometric and meristic characters for *N. ayraudi*. Means with the same letter are not significantly different. Significant differences at 0.05 level are indicated by ***. (see Table 4 for key to character abbreviations).

| | Area | | |
|---|---|---|---|
| Character | 1 | 2 | 3 |
| A. Morphometric | | | |
| CFL | 374.45aa | 393.65aa | 403.05aa |
| SL | 321.80bb | 334.00ab | 352.58aa*** |
| SDF | 180.90aa | 190.80aa | 197.05aa |
| SAF | 192.10aa | 203.65aa | 209.84aa |
| DFL | 89.50aa | 92.08aa | 97.92aa |
| AFL | 0.01aa | 0.01aa | 0.01aa |
| CPW | 0.04aa | 0.04aa | 0.04aa |
| HL | 114.59aa | 120.24aa | 123.49aa |
| HW | 25.27ab | 24.75bb | 27.30aa*** |
| SNL | 87.34bb | 94.48ab | 99.07aa*** |
| HED | 18.64bb | 19.36ab | 19.57aa*** |
| VED | 16.55aa | 16.82aa | 17.33aa |
| MBH | 102.88ab | 95.39bb | 111.48aa*** |
| SPF | 98.46bb | 103.96ab | 108.77aa*** |
| PL | 34.81aa | 36.95aa | 37.01aa |
| B. Meristic | | | |
| NDF | 5.70aa | 5.74aa | 5.70aa |
| NAF | 5.60aa | 5.59aa | 5.60aa |
| NGR | 5.48bb | 5.55aa | 5.53ab*** |
| NV | 4.24aa | 4.24aa | 4.23aa |

Table 4.2b: Summary of multiple range tests of morphometric and meristic characters with significant differences. Mean values increase from left to right. (See text for locations and Table 4.0 abbreviations).

| Character | Area | | | ANOVA |
|---|---|---|---|---|
| A. Morphometric | | | | |
| CFL | 3 | 2 | 1 | $p<0.05$ |
| HW | 3 | 2 | 1 | $p=0.0172$ |
| SNL | 3 | 2 | 1 | $p=0.0176$ |
| VED | 3 | 2 | 1 | $p<0.05$ |
| MBH | 3 | 2 | 1 | $p=0.0147$ |
| SPF | 3 | 2 | 1 | $p=0.0780$ |
| B. Meristic | | | | |
| NGR | 3 | 2 | 1 | $p=0.0181$ |

Table 4.3: Results of principal component analysis of morphometric and meristic characters of ocean jackets

| Character | Factor loadings on eigenvectors | | | | Covariation between eigenvectors & characters | | | |
|---|---|---|---|---|---|---|---|---|
| A. Morphometric | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| CFL | 0.577 | 0.577 | 0.345 | 0.043 | 2561.061 | 2317.405 | 1449.723 | 1552.325 |
| SL | 0.066 | 0.530 | 0.280 | -0.760 | 2317.405 | 2166.315 | 1336.277 | 1429.353 |
| SDF | 0.034 | 0.034 | -0.698 | 0.037 | 1449.723 | 1336.277 | 1052.050 | 1049.053 |
| SAF | 0.042 | 0.368 | -0.505 | 0.011 | 1552.325 | 1429.353 | 1049.053 | 1098.826 |
| DFL | -0.023 | 0.153 | 0.131 | 0.119 | 636.827 | 578.127 | 360.051 | 378.038 |
| AFL | 0.016 | 0.116 | 0.070 | 0.034 | 512.313 | 468.258 | 293.841 | 310.416 |
| CPW | 0.004 | 0.033 | 0.025 | 0.030 | 146.802 | 133.728 | 84.558 | 85.901 |
| HL | 0.018 | 0.171 | 0.094 | 0.355 | 753.047 | 679.969 | 430.380 | 460.733 |
| HW | 0.004 | 0.031 | 0.015 | 0.027 | 136.325 | 124.921 | 79.297 | 84.009 |
| SNL | 0.014 | 0.149 | 0.107 | 0.061 | 653.228 | 598.866 | 367.672 | 397.922 |
| HED | 0.001 | 0.015 | 0.014 | 0.002 | 66.294 | 60.410 | 36.652 | 39.227 |
| VED | 0.001 | 0.012 | 0.001 | 0.051 | 53.022 | 46.313 | 31.556 | 32.909 |
| MBH | 0.993 | -0.110 | -0.001 | 0.009 | 504.033 | 462.200 | 178.099 | 272.672 |
| SPF | 0.013 | 0.157 | 0.123 | 0.304 | 690.237 | 625.354 | 386.761 | 417.656 |
| PL | -0.001 | 0.033 | 0.041 | 0.041 | 144.692 | 130.988 | 78.711 | 82.388 |
| Eigenvalue | 7470.76 | 314.11 | 82.10 | 48.57 | | | | |
| Cumulative % | 93.09 | 97.00 | 98.03 | 98.63 | | | | |
| B. Meristic | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| NDF | 0.203 | 0.725 | 0.102 | -0.651 | 0.692 | 0.398 | 0.119 | 0.005 |
| NAF | 0.165 | 0.619 | 0.115 | 0.759 | 0.398 | 0.563 | 0.089 | -0.011 |
| NGR | 0.940 | -0.215 | -0.265 | 0.011 | 0.119 | 0.089 | 1.454 | -0.008 |
| NV | -0.009 | -0.005 | -0.026 | -0.022 | -0.005 | 0.011 | -0.008 | 0.016 |
| Eigenvalue | 1.54 | 1.00 | 0.62 | 0.22 | | | | |
| Cumulative % | 45.32 | 74.73 | 92.98 | 99.53 | | | | |

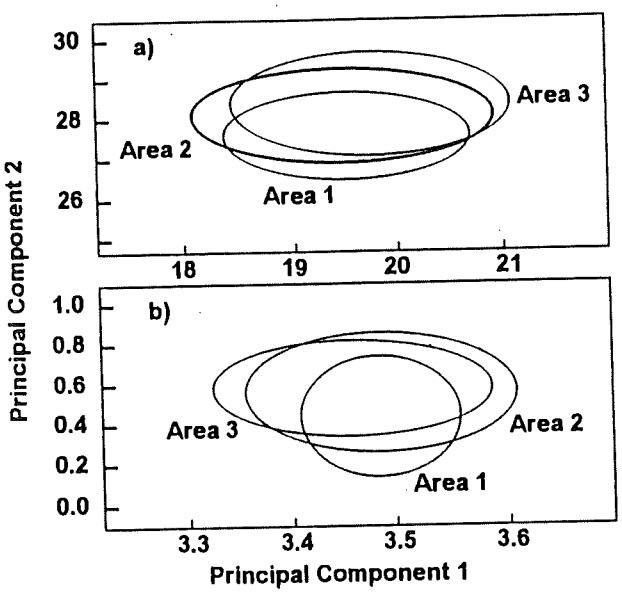


Figure 4.1 Plot of principal component 1 and principal component 2 for (a) morphometric and (b) meristic data.

Table 4.4: Summary of stepwise discriminant analysis for all characters measured. Only discriminated characters (in order of importance) are shown.

| Step | Variable removed entered | Partial R**2 | F Statistic | Prob. > F | Wilks' Lambda | Prob. < Lambda | Average Squared Canonical Correlation | Prob. > ASCC |
|---|---|---|---|---|---|---|---|---|
| **Morphometric** | | | | | | | | |
| 1 | HW | 0.1553 | 5.2400 | 0.0081 | 0.5447 | 0.0081 | 0.0776 | 0.0081 |
| 2 | VED | 0.3992 | 18.6080 | 0.0001 | 0.5074 | 0.0001 | 0.2537 | 0.0001 |
| 3 | SNL | 0.3032 | 11.9660 | 0.0001 | 0.3535 | 0.0001 | 0.3868 | 0.0001 |
| 4 | AFL | 0.1908 | 6.3650 | 0.0033 | 0.2861 | 0.0001 | 0.4509 | 0.0001 |
| 5 | CFL | 0.2359 | 8.1800 | 0.0008 | 0.2186 | 0.0001 | 0.5055 | 0.0001 |
| 6 | SL | 0.2584 | 9.0600 | 0.0004 | 0.1621 | 0.0001 | 0.5500 | 0.0001 |
| **Meristic** | | | | | | | | |
| 1 | NGR | 0.0827 | 2.6140 | 0.0818 | 0.9173 | 0.0818 | 0.0413 | 0.0818 |

Table 4.5. Classification matrix of ocean jackets based on linear discriminant function of morphometric and meristic characters. (Bold numbers indicate correct classifications)

| Area of origin | Percentage classification of population (number of observations) | | | Frequency misclassified % |
|---|---|---|---|---|
| A. Morphometric | | | | |
| | 1 | 2 | 3 | |
| 1 | **95.00(19)** | 0.00(0) | 10(92) | 38.33 |
| 2 | 5.00(1) | **95.00(19)** | 5.00(1) | 31.67 |
| 3 | 21.05(4) | 0.00(0) | **85.00(15)** | 30 |
| B. Meristic | | | | |
| | 1 | 2 | 3 | |
| 1 | **66.67(14)** | 33.33(7) | 0.00(0) | 57.38 |
| 2 | 45.00(9) | **55.00(11)** | 0.00(0) | 42.62 |
| 3 | 60.00(12) | 40.00(8) | **0.00(0)** | 0 |

Table 4.6. Mahalanobis distance between areas based on morphometric and meristic measurements on ocean jackets

| | | Mahalanobis (D2) distance to area | | |
|---|---|---|---|---|
| Area of origin | | 1 | 2 | 3 |
| A. Morphometric | | | | |
| | 1 | | 14.58825 | 4.48137 |
| | 2 | 14.58825 | | 19.67633 |
| | 3 | 4.48137 | 19.67733 | |
| B. Meristic | | | | |
| | 1 | | 0.48587 | 0.22739 |
| | 2 | 0.48587 | | 0.22739 |
| | 3 | 0.22739 | 0.04848 | |