POPULATION STRUCTURE OF ORANGE ROUGHY (*Hoplostethus atlanticus*) IN AUSTRALIAN WATERS.

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SUMMARY AND RECOMMENDATIONS

Nine polymorphic loci were used to investigate the population structure of orange roughy in Australia and New Zealand waters.

The data suggest that orange roughy are divided into three sub-populations. These occur in the broad areas of:-

1. New Zealand
2. Eastern Australia and Tasmania
3. South Australia

Further work should be undertaken before firm conclusions are drawn from the data presented.

The problems of stock delineation in orange roughy is complex. There are many unknowns and it is likely that several different approaches to the problem will yield more useful results than a single study. Like the studies of Ovenden et al. and Lester et al., our study points to population sub-division but none are conclusive.

The allozyme study carried out by us was never intended to be more than a preliminary examination of orange roughy population structure. It was severely constrained by funds and time. Only nine out of approximately 18 polymorphic loci were studied beyond the pilot study. No rare alleles were included so that analyses which utilise these alleles could not be applied to the data.

Further allozyme studies are warranted because knowledge of the genetic structure of the orange roughy "population" is crucial to the proper biological management of the species. Such work needs to be carefully planned to include investigation into spatial and temporal variation and sampling and analysis of juveniles. This work should be supported by further mtDNA studies (these may have already been completed) and possibly nuclear DNA fingerprinting, although this latter technique may yield data too complex to be useful to managers.

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Our thanks also go to Themo Terzis for the adaption of "Doc Holliday" for use with the Macintosh computer, and Rees Griffiths for compiling the Macintosh program to test for inter-population heterogeneity of allelic and genotypic frequencies, and for general computing troubleshooting.

INTRODUCTION

Orange roughy, *Hoplostethus atlanticus*, are known to be distributed in the continental slope waters of the Atlantic and Pacific oceans (Smith, 1986). This species is found at depths ranging from 800 metres to 1200 metres, and in water temperatures between 4^0C to 9^0C. Fish mature at 33 cm to 35 cm in length (weighing about 1.5 kg) and form spawning aggregations during winter (May to July in Australia, July/August in New Zealand). However, the complete life cycle is as yet unknown, and larvae have not yet been described. Life span can only be estimated at 15 to 30 years, as aging methods (eg. otolith growth rings) have proved difficult for this species. Natural mortality rates are not known. The above information is from Williams, 1987.

Prior to the explorations of deepwater, orange roughy was regarded as a relatively uncommon species. The fish are not sufficiently abundant in the north-east Atlantic ocean to support a commercial fishery there (Smith, 1986). However, major deepwater fisheries rapidly developed from the early 1970's in New Zealand (Smith,1986), and later in Australia with the discovery of dense spawning aggregations enabling catch rates in excess of 5 tonnes per hour (Williams, 1987).

Due to escalating industry interest in the resource, a major research effort was initiated by the Australian Commonwealth and southern State governments for the 1987/'88 fiscal year through F.I.R.T.A and F.D.T.A. (Williams, 1987). Conducted by several organisations, the research was primarily directed towards the development of a management strategy that would ensure a sustainable yield of orange roughy, both now and in the future. To do this, a safe level of exploitation of the fish needs to be determined to allow protection of the existing stocks.

In a detailed study of the parasites of Orange Roughy Lester et al. (1988) reported eight separate stocks in Australian and New Zealand waters.

Electrophoresis is a technique which is useful for the delineation of species and species boundaries, for the detection of hybridization, and for the determination of population structure. This technique has been widely applied in fisheries investigations because of the importance of defining subpopulations for informed fishery management. Smith (1986) used isozyme electrophoresis to investigate the structure of the New Zealand population of orange roughy, but he did not include any samples from the Australian fishery in his study. This report details the results of an electrophoretic investigation of orange roughy from Australian waters.

The objectives of our studies on orange roughy were:

1. to determine if Australian orange roughy comprise one or several sub-populations, and to investigate the spatial structure of potential sub-populations, and

2. to compare the orange roughy samples from Australia with a sample taken from the Chatham Rise off the east coast of New Zealand.

METHODS

Sample Collection

Collections of approximately 100 fish each, from 5 localities in Australian Fishing Zone, and 1 locality off the east coast of New Zealand were obtained by the N.S.W. Fisheries Research Institute (Cronulla), NSW Department of Agriculture for an electrophoretic survey of genetic variation in orange roughy. Table 1 and Figure 1 show details of these collections. From NSW both adult and juvenile fish were sampled.

Fish were sent frozen to the laboratory, and were kept at -20^{0}C. Samples of liver, muscle and heart were taken from partially thawed fish and stored cryogenically (-180^{0}C to -196^{0}C) until required for electrophoresis. Large (adult) fish from NSW were sent as head and gut only so that the fillets from these fish could be marketed. Tissue specimens, frozen at -70^{0}C, were obtained from New Zealand. No deterioration in enzyme activity was observed in these samples.

Table1 Collection data for orange roughy (*Hoplostethus atlanticus*).

Collection Site	Collection Date	Location	No. of Animals	Sex Ratio	Size Range LCF (cm)
New Zealand	15/2/88	43°S,175°30'W	50	20M:30F:0J	20.3-36.3
Kangaroo Is.S.A.	2/88	37°20'S,138°40'E	94	66M:27F:1J	31.5-49.0
Portland Vic.	2/88	38°37'S,141°03'E	70	16M:54F:0J	31.8-41.2
South NSW-1	11/87	-	11	7M:4F:0J	15.2-29.2
South NSW-2	6/4/88	-	62	15M:47F:0J	33.1-40.9
South NSW-3	4-5/88	35°S,151°E	107	9M:23F:75J	16.0-32.0
West Tasmania	4/88	42°48'S,144°52'E	94	41M:49F:4J	32.0-43.4
East Tasmania	2,3/2/88	41°38'S,148°40'E	99	69M:21F:1J	32.0-42.5

Key: M = male, F = female, J = juvenile.
South NSW-3 = pooled collections from:- Kiama, Shoalhaven, Jervis Bay, and Ulladulla.

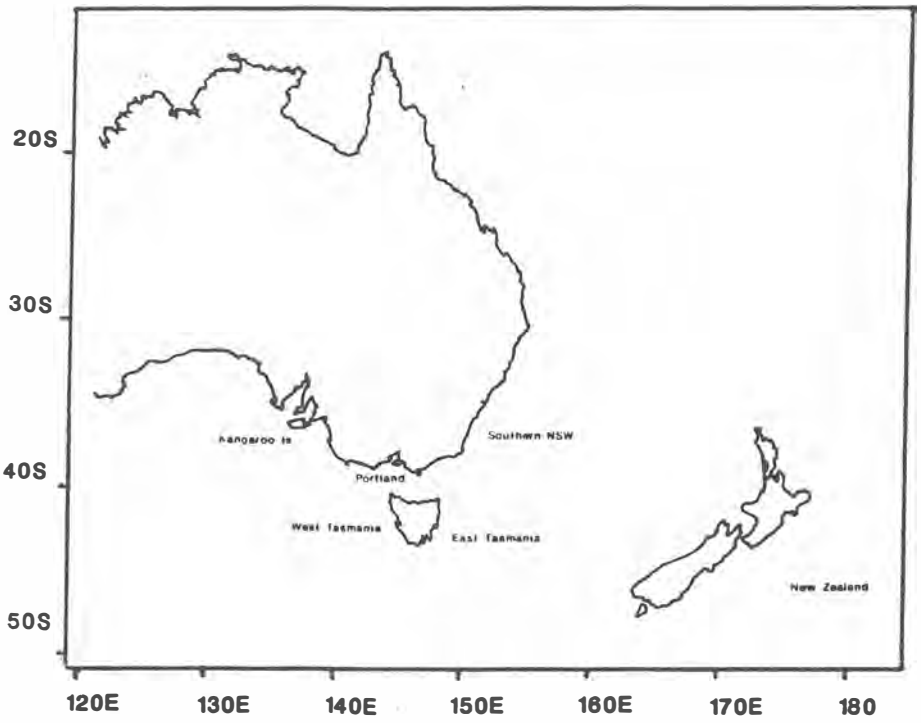

Figure 1 Map to show the collection sites of orange roughy.

Electrophoresis

Full details of the electrophoretic procedures used in this study follow the methods described in the whiting stock identification report (FIRTA 83/16). Starch gels were made from 12% (w/v) Electrostarch (Lot Number 87). Due to time restrictions on this study, cellogel (Chemetron, Milan) was not investigated as a support matrix for orange roughy.

50 enzymes representing 60 presumptive loci were surveyed for genetic variation using horizontal starch gel electrophoresis for three different tissues (heart, liver and muscle). Initially, tissue samples were divided and homogenised in both distilled water and homogenising buffer to screen for a technique which provided the maximum activity and resolution on the gels. Distilled water proved to be satisfactory for orange roughy. Appendix 1 details the enzymes surveyed for genetic variation, together with electrophoresis running conditions and presumed number of loci for each tissue.

Eleven enzyme loci were found to be polymorphic, namely: Aat, Acon, Ak, Est, Gpi-1, Gpi-2, Idh, Mdh, Me, Mpi and Pgm. Only those loci with patterns of variation that were consistent with the known subunit structure of the enzyme (Shaklee and Keenan, 1986) and/or displayed a phenotype distribution in Hardy/Weinberg equilibrium were used for the population analysis. Thus, nine loci were selected for detailed analysis. These loci were:- Aat, Est, Gpi-1, Gpi-2, Idh-2, Mdh, Me, Mpi and Pgm, and are detailed in Table 2. Mdh was included for analysis even though this locus did not fit Hardy-Weinberg equilibrium, as this disequilibrium was due to the absence of two phenotypes 'ac' and 'ad' (see Appendix 2), and the heterozygosity values were non-significant (see Appendix 3).

Table 2 Polymorphic loci used in the population study of orange roughy.

ENZYME NAME	EC NUMBER	LOCUS	SUBUNIT STRUCTURE	TISSUE	BUFFER
Aspartate aminotransferase	EC 2.6.1.1	Aat	dimer	liver	Cam pH6.1
Esterase	EC 3.1.1.1	Est	monomer	liver	Cam pH6.1
Glucosephosphate isomerase	EC 5.3.1.9	Gpi-1	dimer	muscle	TC pH5.8
		Gpi-2	dimer	muscle	TC pH5.8
Isocitrate dehydrogenase	EC1.1.1.42	Idh	dimer*	heart	TC pH6.8
Malate dehydrogenase	EC 1.1.1.37	Mdh	dimer*	heart	TC pH6.8
Malic enzyme	EC 1.1.1.40	Me	tetramer*	liver	Cam pH6.1
Mannosephophate isomerase	EC 5.3.1.8	Mpi	monomer	heart	TC pH6.8
Phosphoglucomutase	EC 5.4.2.2	Pgm	monomer	muscle	TC pH5.8

KEY: * = atypical heterozygotes observed (see Appendix 2).

Data analysis

Names of enzymes and Enzyme Commission (EC) numbers follow the recommendations of the Commission on Biochemical Nomenclature (Anon, 1984). For multi-locus 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 program, SEPBOTH, performs G-statistic (Sokal and Rohlf, 1981) comparisons between all possible pairs of OTUs (operational taxonomic units), with the level of significance set at 99%. The population structure of orange roughy was examined by G-statistic comparisons of allele frequencies between locations in Australia, and one locality in New Zealand.

The program SEPBOTH was adapted from DOC HOLLIDAY (Holliday, 1987) for use with the Macintosh computer.

We used a continuous character, maximum likelihood method for constructing phylogenetic trees from these allele frequency data. The program CONTML (version 3.02), is part of Felsenstein's (1981, 1982) PHYLIP package. The program CONPLOT uses the output from CONTML to plot a dendrogram.

Genotype and allele frequencies were examined for internal consistency with the Hardy-Weinberg distribution using the G-statistic, and for the goodness-of-fit of heterozygosity values (H_L, and $H_{L(obs)}$) using Chi-squared (Pamilo and Varvio-Aho, 1984), and for inter-population heterogeneity using the chi-square test.

RESULTS AND DISCUSSION

We have examined the population structure of orange roughy throughout its known Australian range, using horizontal starch gel electrophoresis to study nine polymorphic enzyme loci (Aat, Est and Me from liver, Gpi-1, Gpi-2 and Pgm from muscle, and Idh, Mdh and Mpi from heart). The enzyme banding patterns for these loci are described in Appendix 2.

Smith (1986) published data on orange roughy from New Zealand waters utilising more polymorphic loci. Those loci not included in our study were:-Adh-1, Gpdh-1, Gpdh-2, G6pdh, Idh-1, Ldh-1, Ldh-2, and Pgdh. All of these loci were not detected as polymorphic in our pilot screening (see Appendix 1), and showed a low level of polymorphism in Smith's data. Data from the Mpi locus was not included in Smith's study.

At the time of sampling, southern NSW was the only locality in Australia where juvenile orange roughy were known to occur. Both "large" and "small" fish were sampled. Figure 2 shows the bimodal length frequency distribution of fish from these collections. This sample was subdivided in the following way:-

NSWLarge = adult fish >30cm (sex determinable),

NSWSmall = juvenile fish <30cm (no gonad development).

Fish falling into the 30 cm size class were deleted from these groupings, as some, but not all, of these fish showed gonad development (sex determinable). Furthermore, the juvenile fish were subdivided into two groups, according to broad geographical areas, in the following way:-

NSWSmall-1 = collections from the Shoalhaven, Jervis Bay, and Kiama,

NSWSmall-2 = fish collected from Ulladulla.

The sample from the Chatham Rise, east New Zealand, consisted generally of smaller fish (see Figure 3), but, because of the small sample size, large and small were analysed together. Despite this size difference, all fish were allocated a sex by the collectors (refer to Table 1).

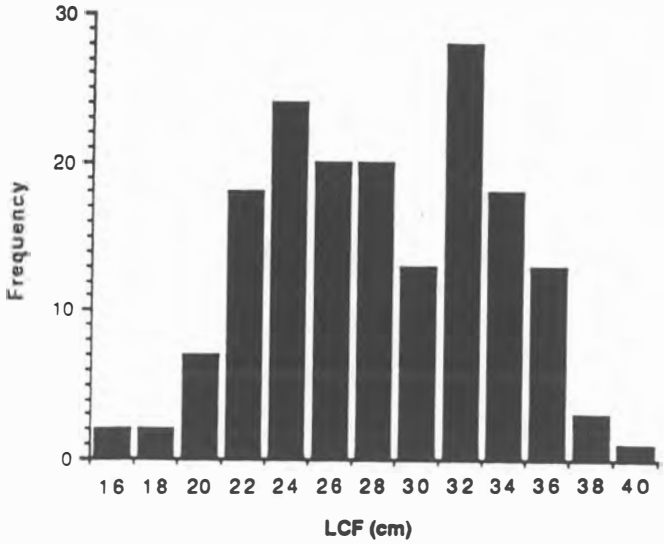

Figure 2 Frequency distribution (LCF) of orange roughy collected from southern NSW.

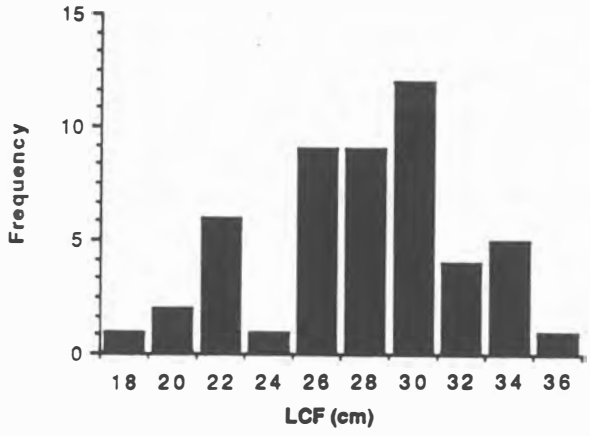

Figure 3 Frequency distribution (LCF) of orange roughy collected from the Chatham Rise, east New Zealand.

Allele frequencies, numbers of animals successfully scored, and heterozygosity values for each polymorphic locus at each locality, together with the divided adult and juvenile classes, are presented in Appendix 3, Table 3.1.

Goodness-of fit to the Hardy-Weinberg distribution were calculated for each population for each locus. Mdh was consistently out of equilibrium for all but two samples (New Zealand and NSWSmall-2), however, heterozygosity values were non-significant, so this locus was included for further analyses. The sample from east Tasmania was out of Hardy-Weinberg equilibrium at 5 loci :- Aat, Idh, Mdh, Mpi, and Pgm. For Aat and Idh the heterozygosity values were also significant, indicating a possible mixing of populations within this collection. For any future sampling of the east coast of Tasmania, care should be taken in the labeling of each 'shot', so that the data may be subdivided accordingly.

Inter-population heterogeneity in genotype frequencies were tested for using Chi-squared, see Table 3, below. All loci tested were significantly heterogeneous, except for Gpi-2.

Table 3 Genic contingency Chi-square tests for heterogeneity across all samples of orange roughy, by locus.

Locus	No. of alleles	Σ N	χ^2	d.f		Probability
Gpi-2	5	843	49.928	36		0.1>P>0.05
Gpi-1	5	819	61.523	36	*	0.01>P>0.005
Pgm	3	726	43.069	18	*	P>0.005
Est	6	818	79.917	45	*	P>0.005
Mpi	3	626	61.881	18	*	P>0.005
Aat	3	645	57.678	18	*	P>0.005
Me	2	713	69.685	9	*	P>0.005
Idh	4	815	126.469	27	*	P>0.005
Mdh	4	749	62.709	27	*	P>0.005

Key: * = significant heterogeneity

Allele frequencies were analysed by pairwise comparison, using the G-statistic, to determine if the population was subdivided geographically (see Appendix 3, Table 3.2). Significant differences were found between the adult and all juvenile classes, except NSWSmall-2 (Ulladulla). Furthermore, significant differences were also found between all localities sampled (35/43 comparisons). To gain more information on the nature of population substructuring in orange roughy, G-tests were performed on each locus individually. Tables 4-12 are summaries of these G-test results in triangle form, and Figures 4-12 are maps to show the allele frequencies as pie charts.

For Aat, there were significant differences between 16 out of 43 comparisons (see Table 4), separating orange roughy into 4 subgroups:-

1. New Zealand,
2. NSW adult fish,
3. NSW juvenile fish, east Tasmania, and Victoria,
4. west Tasmania and South Australia (see Figure 4).

Table 4 Summary of the results of the G-tests on Aat allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand	*						*	
2	EastTasman			*		*			
3	WestTasman						*	*	
4	KangIsSA				*		*	*	*
5	PortlandV								
6	NSWLarge					*			
7	NSWSmall						*	*	*
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

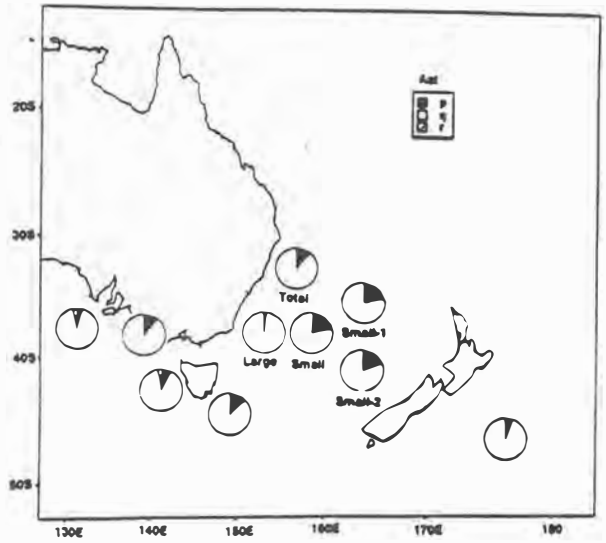

Figure 4 Map to show Aat allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Est, there were significant differences between 8 out of 43 comparisons (see Table 5), separating orange roughy into 2 subgroups:-

1. New Zealand, eastern Australia and Tasmania,
2. South Australia (see Figure 5).

Table 5 Summary of the results of the G-tests on Est allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand			*					
2	EastTasman						*	*	
3	WestTasman								
4	KangIsSA					*	*	*	
5	PortlandV								
6	NSWLarge								
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

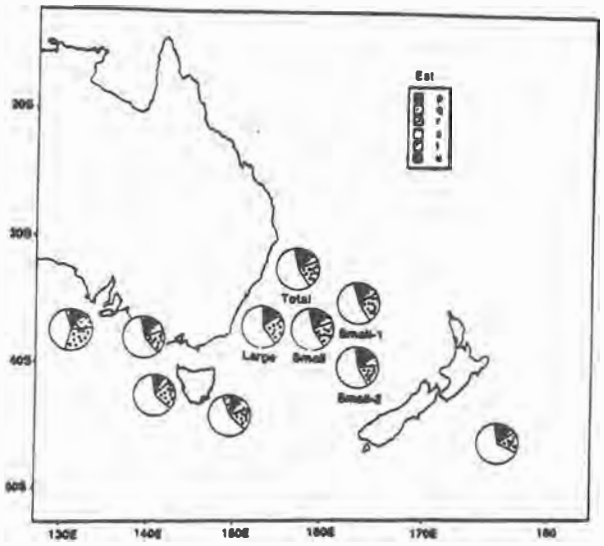

Figure 5 Map to show Est allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Gpi-1, significant differences were found between 3 out of 43 comparisons (see Table 6), weakly separating orange roughy into 2 subgroups:-

1. New Zealand, eastern Australia and Tasmania,
2. South Australia (see Figure 6).

Table 6 Summary of the results of the G-tests on Gpi-1 allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand								
2	EastTasman								
3	WestTasman								
4	KangIsSA				*				
5	PortlandV						*		
6	NSWLarge								
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

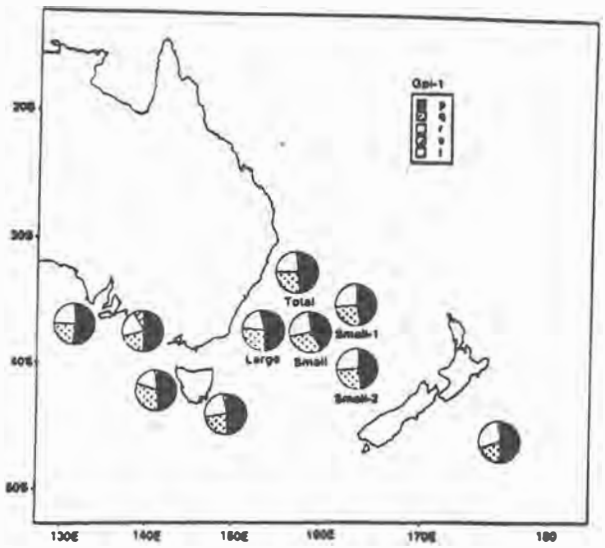

Figure 6 Map to show Gpi-1 allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Gpi-2, although heterogeneous (see Table 3), there were significant differences between 4 out of 43 comparisons (see Table 7), separating orange roughy into 2 subgroups:-

1. New Zealand, eastern Australia and Tasmania,
2. South Australia (see Figure 7).

Table 7 Summary of the results of the G-tests on Gpi-2 allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand								
2	EastTasman								
3	WestTasman								
4	KangIsSA						*	*	*
5	PortlandV								
6	NSWTotal								
7	NSWLarge								
8	NSWSmall								
9	NSWSmall-1								
10	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

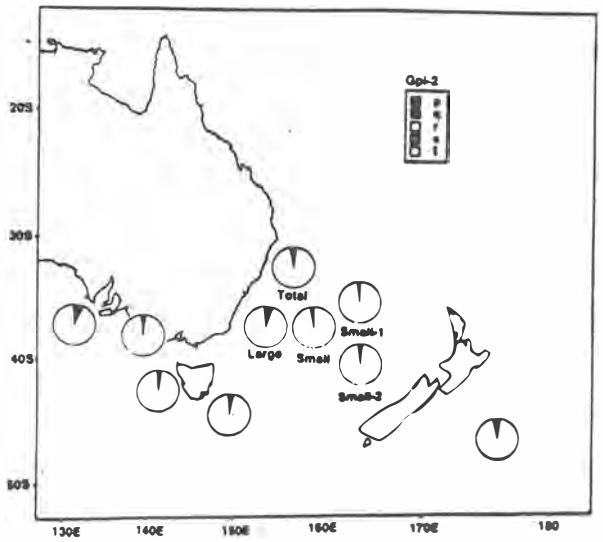

Figure 7 Map to show Gpi-2 allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Idh, there were significant differences between 19 out of 43 comparisons (see Table 8), separating orange roughy into 4 subgroups:-

1. New Zealand,
2. NSW adult fish, and NSW juvenile fish from Ulladulla,
3. NSW juvenile fish from the Shoalhaven (& etc), and Tasmania,
4. Victoria and South Australia (see Figure 8).

Table 8 Summary of the results of the G-tests on Idh allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand	*	*	*			*	*	
2	EastTasman			*		*			
3	WestTasman			*	*	*			*
4	KangIsSA					*	*	*	
5	PortlandV								
6	NSWLarge						*	*	
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

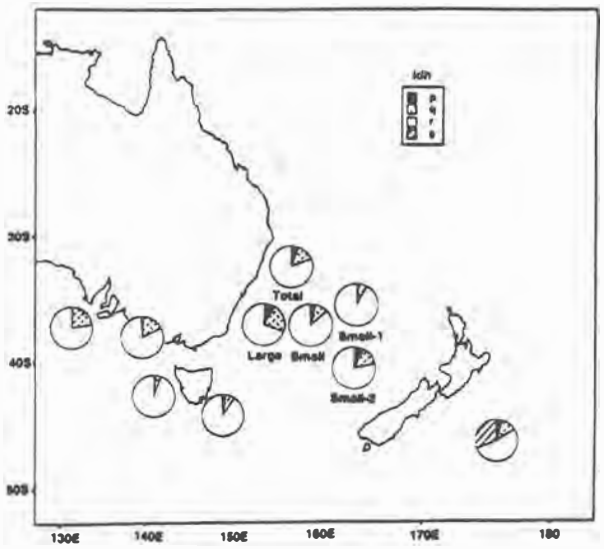

Figure 8 Map to show Idh allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Mdh, there were significant differences between 15 out of 43 comparisons (see Table 9), separating orange roughy into 3 subgroups:-

1. New Zealand,
2. NSW adult and juvenile fish, Victoria and Tasmania,
4. South Australia (see Figure 9).

Table 9 Summary of the results of the G-tests on Mdh allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand	*	*	*	*	*	*	*	
2	EastTasman			*					
3	WestTasman			*					
4	KangIsSA					*	*	*	*
5	PortlandV								
6	NSWLarge								
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

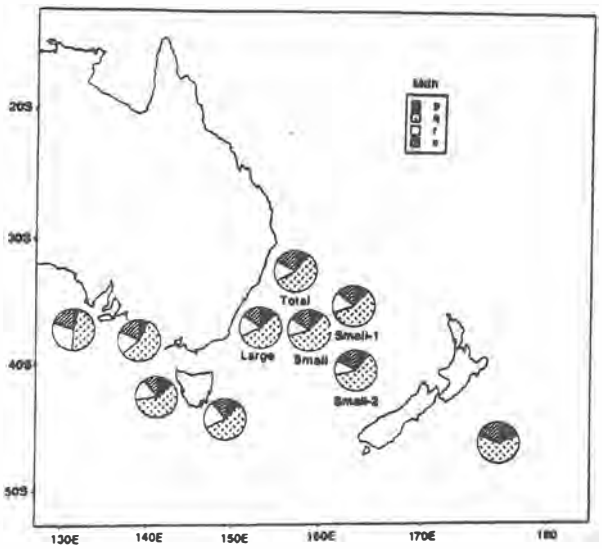

Figure 9 Map to show Mdh allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Me, there were significant differences between 9 out of 43 comparisons (see Table 10), separating orange roughy into 2 subgroups:-

1. New Zealand, eastern Australia and Tasmania,
2. South Australia (see Figure 10).

Table 10 Summary of the results of the G-tests on Me allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand			*					
2	EastTasman			*					
3	WestTasman			*					
4	KangIsSA				*	*	*	*	*
5	PortlandV								
6	NSWLarge								
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

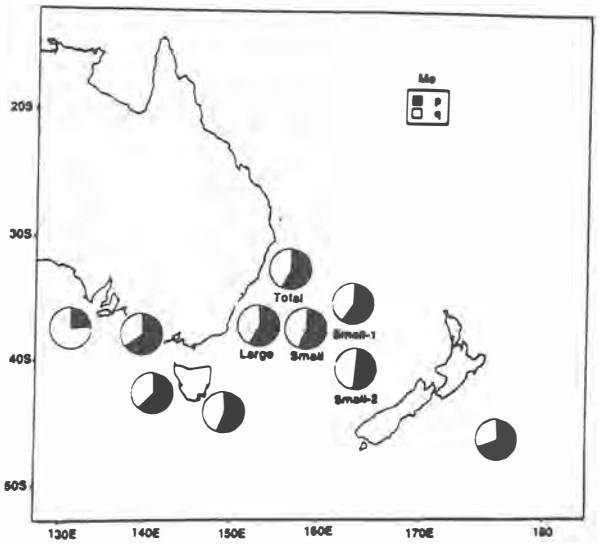

Figure 10 Map to show Me allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Mpi, there were significant differences between 14 out of 43 comparisons (see Table 11), weakly separating orange roughy into 5 subgroups:-

1. New Zealand,
2. NSW juvenile fish from Ulladulla (NSWSmall-2),
3. NSW adult fish, NSWSmall-1, Victoria, and east Tasmania,
4. west Tasmania, and 5. South Australia (see Figure 11).

Table 11 Summary of the results of the G-tests on Mpi allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand	*	*	*	*	*			*
2	EastTasman								
3	WestTasman			*			*	*	*
4	KangIsSA								
5	PortlandV						*		*
6	NSWLarge								
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

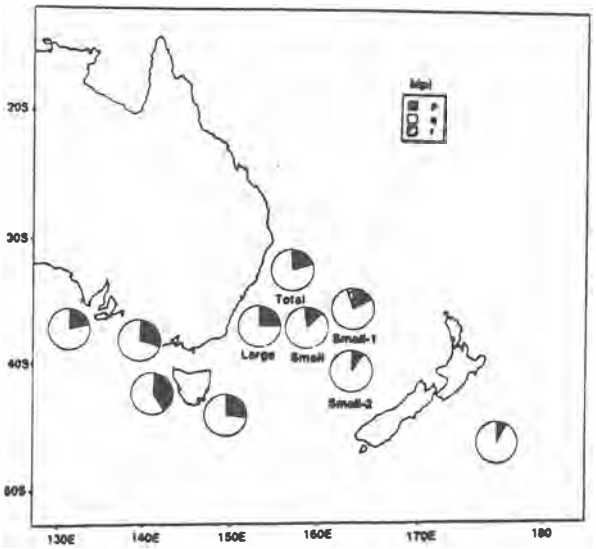

Figure 11 Map to show Mpi allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

For Pgm, there were significant differences between 4 out of 43 comparisons (see Table 12), weakly separating orange roughy into 3 subgroups:-

1. New Zealand, NSW adult fish, NSW juvenile fish from Ulladulla (NSWSmall-2), Victoria, west Tasmania, and South Australia,
2. NSW juvenile fish from the Shoalhaven & etc (NSWSmall-1), and 3.. east Tasmania (Figure 12).

Table 12 Summary of the results of the G-tests on Pgm allele frequencies in triangle form.

		2	3	4	5	6	7	8	9
1	NewZealand								
2	EastTasman						*	*	
3	WestTasman								
4	KangIsSA								
5	PortlandV								
6	NSWLarge								
7	NSWSmall								
8	NSWSmall-1								
9	NSWSmall-2								

Key: * = Significant G-test, at the 99% level.

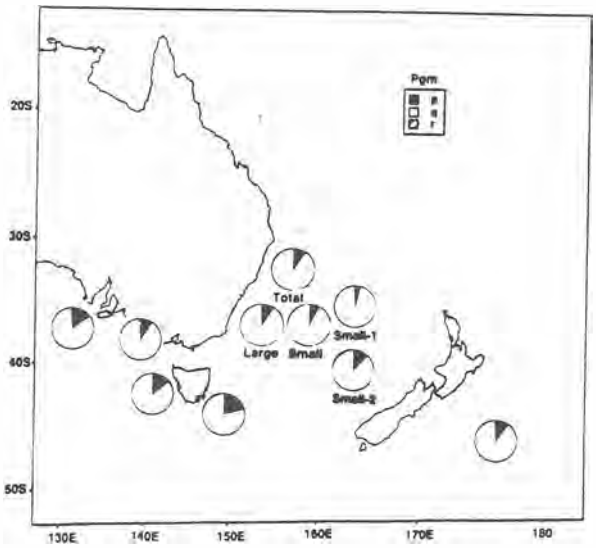

Figure 12 Map to show Pgm allele frequencies, of orange roughy samples in the Australian collections, as pie charts.

The allele frequency data was also used to construct a dendrogram to show the genetic relationships between the localities sampled. We used Felsenstein's (1981,1982) CONTML program to construct the maximum likelihood phylogenetic tree (see Figure 13). The dendrogram is clustered geographically.

Considering all the above information from all the G-tests and the dendrogram, 3 subpopulations of orange roughy are indicated:-

1. New Zealand,
2. eastern Australia and Tasmania,
3. South Australia.

The sample from the Chatham Rise, east New Zealand is separated by 4 loci:- Aat (Figure 4), Idh (Figure 8), Mdh (Figure 9), and Mpi (Figure 11), and by 25 comparisons across all loci (see Table 13).

South Australia is separated at 6 loci:- Est (Figure 5), Gpi-1 (Figure 7), Mdh (Figure 9), Me (Figure 10), and Mpi (Figure 11), and by 43 comparisons across all loci (see Table 12). The dendrogram (Figure 13) separates New Zealand and South Australia with longer branch lengths relative to the other localities.

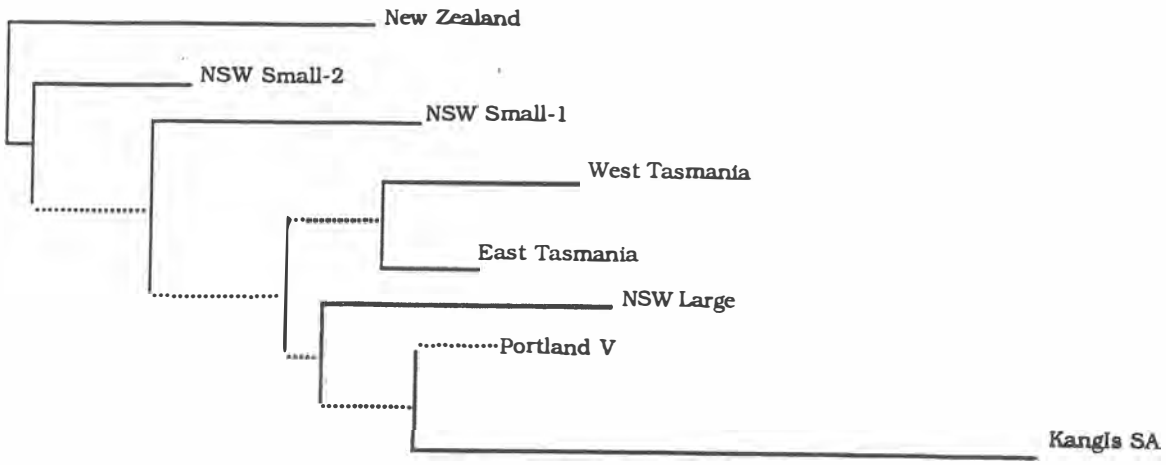

Figure 13 Dendrogram to show the relationships between orange roughy samples in the Australian collections (CONTML plot).

The large number of significant G-tests between the other localities (refer to Table 13) indicates a complex relationship within the orange roughy that is not readily pinpointed as a perturbance at any one locality (refer to Tables 4-12). In view the strict geographic manner in which the dendrogram was drawn, the isolation-by-distance model (Richardson, 1986) should be considered. This model allows gradual, geographically-related, changes to occur in allele frequencies, with the effects at each locus being independent, and no geographical discontinuities in allele frequency.

Table 13 Summary of significant differences across all loci for each locality sampled.

	Aat	Est	Gpi-1	Gpi-2	Idh	Mdh	Me	Mpi	Pgm	Total
New Zealand	2	1	-	-	6	8	1	7	-	25
E. Tasmania	3	3	-	-	3	2	1	1	3	16
W. Tasmania	3	-	-	-	6	2	1	6	-	18
Kang.Is SA	6	5	1	4	7	8	9	2	1	43
Portland Vic	1	-	3	-	1	1	1	3	-	10
NSW Total	3	2	1	1	3	2	1	2	2	17
NSW Large	5	1	-	-	5	2	1	1	-	15
NSW Small	3	2	1	1	3	2	1	2	1	16
NSW Small-1	4	2	-	1	3	2	1	1	1	15
NSW Small-2	2	-	-	1	1	1	1	3	-	9

Allele frequencies of the polymorphic loci are presented by latitude and longitude in Appendix 4, Figures 4.1-4.12. Continuous clines across all localities are not apparent. A number of discontinuous clines support the separation of New Zealand and South Australia into separate subpopulations. For New Zealand these are:- Aat^{q} (Figure 4.1a&b), Est^{q} (Figure 4.2a), Idh^{q} (Figure 4.5a&b), Idh^{r} (Figure 4.6a&b), Mdh^{p} (Figure 4.7a&b), Mdh^{r} (Figure 4.9a&b), Me (Figure 4.10b), Mpi^{p} (Figure 4.11a&b), and Mpi^{q} (Figure 4.12a&b). For South Australia these are:- Aat^{q} (Figure 4.1b), Est^{r} (Figure 4.3a&b), Est^{s} (Figure 4.4a&b), Mdh^{q} (Figure 4.8a&b), Mdh^{r} (Figure 4.9a&b), Me^{p} Figure 4.10a&b), Mpi^{p} (Figure 4.11a&b), and Mpi^{q} (Figure 4.12a&b).

Fluctuations, indicating some separation between east and west Tasmania, occur across 4 alleles:- Est^{q} (Figure 4.2a&b), Mdh^{r} (Figure 4.9a&b), Mpi^{p} (Figure 4.11a&b), and Mpi^{q} (Figure 4.12a&b). This supports the preliminary findings of Ovenden et al. (1989) who reported differences in Orange Roughy from eastern and western Tasmania based on mtDNA restriction site variation. Victoria follows the pattern of other sites clustering either with Tasmania or NSW. NSW juveniles separate at Aat^{q} (Figure 4.1a), Idh^{r} (Figure 4.6a&b), Mpi^{p} (Figure 4.11a&b) and Mpi^{q} (Figure 4.12a&b).

Before any further conclusions can be drawn from these data, more biological information, for example age and growth rate, migration distances and temporal variations in gene frequencies is imperative.

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APPENDIX 1 Details of enzymes surveyed for genetic variation in orange roughy.

Table 1.1 Enzymes investigated in orange roughy.

Enzyme	Abbreviation	Enzyme Commission Number
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
Aldehyde oxidase	Ao	EC 1.2.3.1
Aldehyde dehydrogenase	Aldh	EC 1.2.1.3
Aspartate aminotransferase	Aat	EC 2.6.1.1
Creatine kinase	Ck	EC 2.7.3.2
Diaphorase	Dia	EC 1.6.2.2
D-Amino acid oxidase	Damox	EC 1.4.3.3
D-Aspartate oxidase	Dasox	EC 1.4.3.1
Enolase	Enol	EC 4.2.1.11
Esterase	Est	EC 3.1.1.1
Fructose diphosphatase	Fdp	EC 3.1.3.11
Fumarase	Fum	EC 4.2.1.2
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
Glutamate dehydrogenase	Glud	EC 1.4.1.3

Table 1.1 continued .

Enzyme	Abbreviation	Enzyme Commission Number
Glyceraldehyde-3-phosphate dehydrogenase	Ga3pdh	EC 1.2.1.12
alpha-glycerophosphate dehydrogenase	Gpd	EC 1.1.1.8
Galactose dehydrogenase	Galdh	EC 1.1.1.48
Glyoxylase I	GloI	EC 4.4.1.5
Glyoxylase II	GloII	EC 3.1.2.6
Glycerol dehydrogenase	Glydh	EC 1.1.1.6
Guanine deaminase	Gda	EC 3.5.4.3
Hexokinase	Hk	EC 2.7.1.1
Hexosaminidase	Hex	EC 3.2.1.52
beta-Hydroxybutyrate dehydrogenase	Hbdh	EC 1.1.1.30
Isocitrate dehydrogenase	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
Mannosephosphate isomerase	Mpi	EC 5.3.1.8
Nucleoside phosphorylase	Np	EC 2.4.2.1
Peptidases (A, B, C, D, S)	Pep	EC 3.4.11
Phosphoglucomutase	Pgm	EC 5.4.2.2
6-Phosphogluconate dehydrogenase	Pgd	EC 1.1.1.44
Pyruvate kinase	Pk	EC 2.7.1.4
Sorbitol dehydrogenase	Sdh	EC 1.1.1.14
Superoxide dismutase	Sod	EC 1.15.1.1
Xanthine dehydrogenase	Xdh	EC 1.1.1.204

A total of 50 enzyme systems investigated.

Table 1.2 Enzymes studied, tissues investigated, electrophoresis running conditions and presumed number of loci for orange roughy.

Key: **L** = liver, **M** = muscle, **H** = heart, ^{S} = used in Smith's study(1986),
* = best tissue/buffer/support matrix for this enzyme,
1 = Tris- EDTA-boric acid pH 9, **2** = Poulik, **3** = Tris-maleate pH 7.8,
4 = Tris-citric acid pH 6.8, **5** = Tris-citric acid pH 5.8,
6 = Citric acid-aminopropyl-morpholine pH 6.1,
ST = Electrostarch gel -Lot Number 87 (Madison, Wisconson.),
a. = anodal migration, **c.** = cathodal migration,
P = polymorphic.

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Acph	L	3,4*,5,6.	ST	1	streaks anodally, ?P
	M	3,5,6*.	ST	1	poor resolution
	H	3,5,6*.	ST	1	poor resolution
Acon	L^{S}	2,5,6*.	ST	1	good activity, P
	M	2,5,6.	ST	-	no activity
	H	2*,5,6.	ST	1	good activity
Ada	L	2,3,5*,6.	ST	1	poor activity
	M^{S}	2*,3,5,6.	ST	1	poor resolution, ?P
	H	2,3*,5,6.	ST	1	poor activity, ?P
Ak	L	3,5,6*.	ST	1	sub-banding, P
	M	5,6*.	ST	1	good activity
	H	5,6*.	ST	1	good activity
Adh	L^{S}	2*,3,5,6.	ST	1	good activity
	M	2*,3,5,6.	ST	1	poor activity
	H	2,3,5,6.	ST	-	no activity

Table 1.2 continued

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Ald	L	3,6.	ST	-	no activity
	M	3,6.	ST	-	no activity
	H	3,6.	ST	-	no activity
Alkph	L	3*,5,6.	ST	1	poor activity
	M	3,5,6.	ST	-	no activity
	H	3*,5,6.	ST	1	poor activity
Ao	L	3,6.	ST	-	no activity
	M	3,6.	ST	-	no activity
	H	3,6.	ST	-	no activity
Aldh	L	3,6.	ST	-	no activity
	M	3,6.	ST	-	no activity
	H	3,6.	ST	-	no activity
Aat	L^s	2,3,5*.	ST	2a&c.	fair activity, P
	M	2*,3,5.	ST	2	poor activity
	H	2*,3,5.	ST	2	fair activity,P
Ck	L	2,3*,5,6.	ST	1	poor resolution
	M	2,3*,5,6.	ST	1	poor resolution
	H	2*,3,5,6.	ST	1	poor resolution
Damox	L	3,5.	ST	-	no activity
	M	3,5.	ST	-	no activity
	H	3,5.	ST	-	no activity
Dasox	L	3,5.	ST	-	no activity
	M	3,5.	ST	-	no activity
	H	3,5.	ST	-	no activity

Table 1.2 continued

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Dia	L	2*,3,5.	ST	1	poor resolution
	M	3,5.	ST	-	no activity
	H	3,5.	ST	-	no activity
Enol	L	6.	ST	-	no activity
	M	6.	ST	-	no activity
	H	6.	ST	-	no activity
Est	L^S	2*,3,5,6.	ST	1	good resolution,P
	M	2,3,5,6.	ST	-	no activity
	H	2,3*,5,6.	ST	1	poor resolution
Fdp	L	3*,6.	ST	1	poor activity
	M	3,6.	ST	-	no activity
	H	3*,6.	ST	1	poor activity
Fum	L	5,6.	ST	-	no activity
	M	5,6.	ST	-	no activity
	H	5,6.	ST	-	no activity
Gda	L	2,3,6.	ST	-	no activity
	M	2,3,6.	ST	-	no activity
	H	2,3,6.	ST	-	no activity
Gdh	L	3,6.	ST	-	no activity
	M	3,6.	ST	-	no activity
	H	3,6.	ST	-	no activity
G6pdh	L^S	2,5*,6.	ST	1	poor resolution
	M	2,5,6.	ST	-	no activity
	H	2,5*,6.	ST	1	poor resolution

Table 1.2 continued

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Gpi	L^s	1*,2,3,5,6.	ST	1	overstains, P
	M^s	1,2,3,5*,6.	ST	2	overstains, P
	H	1,2,3,5*,6.	ST	2	overstains, P
Glud	L	3.	ST	-	no activity
	M	3.	ST	-	no activity
	H	3.	ST	1	poor activity
Glydh	L	5.	ST	-	no activity
	M	5.	ST	-	no activity
	H	5.	ST	-	no activity
Ga3pdh	L	2*,3,5,6.	ST	1	poor activity ?P
	M	2,3,5,6.	ST	2a.&c.	poor activity
	H	2,3,5,6*.	ST	2a.&c.	reasonable activity
Gpd	L	3,5,6.	ST	-	no activity
	M	3,5,6.	ST	-	no activity
	H	3,5,6.	ST	-	no activity
Galdh	L	3,6.	ST	-	no activity
	M	3,6.	ST	-	no activity
	H	3,6.	ST	-	no activity
GloI	L	6	ST	-	no activity
	M	6	ST	-	no activity
	H	6	ST	-	no activity
GloII	L	5	ST	-	no activity
	M	5	ST	-	no activity
	H	5	ST	-	no activity

Table 1.2 continued

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Hk	L	6	ST	-	no activity
	M	6	ST	-	no activity
	H	6	ST	-	no activity
Hex	L	6	ST	1	poor resolution
	M	6	ST	1	poor resolution
	H	6	ST	1	poor resolution
Ibdh	L	5,6.	ST	-	no activity
	M	5,6.	ST	-	no activity
	H	5,6.	ST	-	no activity
Idh	L^s	2,3,5*,6.	ST	1	good activity P
	M	2,3,5,6.	ST	-	no activity
	H^s	2,3,5,6*.	ST	2	good activity, P
Ldh	L	2,3,5*,6.	ST	3	overstains
	M^s	2,3,5*,6.	ST	1	good activity
	H^s	2,3,5*,6.	ST	2	overstains
Lap	L	6	ST	-	no activity
	M	6	ST	-	no activity
	H	6	ST	-	no activity
Mdh	L	2,3,4*,5,6.	ST	1	good activity
	M^s	2*,3,5,6.	ST	1	good activity, ?P
	H^s	2,3,4*,5,6.	ST	1	good activity, P
Me	L	2*,3,4,5,6.	ST	1	poor activity,P
	M	2*,3,5,6.	ST	1	poor activity, ?P
	H^s	2,3,4*,5,6.	ST	1	good activity

Table 1.2 continued

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Mpi	L	2,3,4*,5,6.	ST	1	reasonable activity,P
	M^s	2,3,5,6.	ST	-	no activity
	H^s	2,3,4*,5,6.	ST	1	good activity, P
Np	L	3*,5.	ST	1	good activity
	M^s	3,5.	ST	-	no activity
	H^s	3*,5.	ST	1	good activity
PepA	L	2*,3,5,6.	ST	1	poor resolution,P
	M	2,3,5,6.	ST	-	no activity
	H^s	2*,3,5,6.	ST	1	good activity, P
PepB	L	2*,3,5,6.	ST	1	poor resolution,P
	M	2*,3,5,6.	ST	1	good activity,?P
	H^s	2*,3,5,6.	ST	1	poor activity
PepC	L	2*,3,5,6.	ST	1	poor resolution,P
	M	2,3,5,6*.	ST	-	poor resolution
	H^s	2*,3,5,6.	ST	1	reasonable activity
PepD	L	2,3,5*,6.	ST	1	poor activity
	M	2,3,5,6*.	ST	1	reasonable activity
	H^s	2,3,5,6*.	ST	2	good activity,P
PepS	L	2,3,5,6.	ST	1	poor activity
	M	2,3,5,6*.	ST	1	poor activity?P
	H	2*,3,5,6.	ST	1	poor activity
Pgm	L	2*,3,5,6.	ST	2	good activity,P
	M^s	2*,3,5,6.	ST	1	good activity, P
	H	2,3*,5,6*.	ST	2	good activity,P

Table 1.2 continued

Enzyme	Tissue	Buffer(s)	Support matrix	Presumed no. of loci	Comments
Pgd	L^s	1,2,3,6*.	ST	1	poor activity,P
	M^s	2*,3,6.	ST	-	no activity
	H^s	2,3*,6*.	ST	1	poor activity,P
Pk	L	3,5.	ST	1	poor activity
	M	3,5.	ST	1	poor activity
	H	3,5.	ST	1	poor activity
Sdh	L	3,5,6.	ST	-	no activity
	M	3,5,6.	ST	-	no activity
	H	3,5,6.	ST	-	no activity
Sod	L	3*,5.	ST	2a.&c.	good activity
	M	3*,5.	ST	2a.&c.	good activity
	H	3*,5.	ST	2a.&c.	good activity
Xdh	L	1,3,5,6.	ST	-	poor activity
	M	3,5,6.	ST	-	no activity
	H	3,5,6.	ST	-	no activity

APPENDIX 2 Description of the observed enzyme banding patterns of the polymorphic loci used for the analysis of orange roughy population structure.

Aspartate aminotransferase (Aat) EC 2.6.1.1

Aat was examined in extracts of liver tissue, and migrates anodally in Cam pH 6.1 buffer.

Subunit structure: dimeric

Observed banding pattern: one common (bb) and two rare alleles were detected. Activity was often poor, as was the resolution of the bands. Heterozygotes were mostly detected as difuse zones of activity as shown below:-

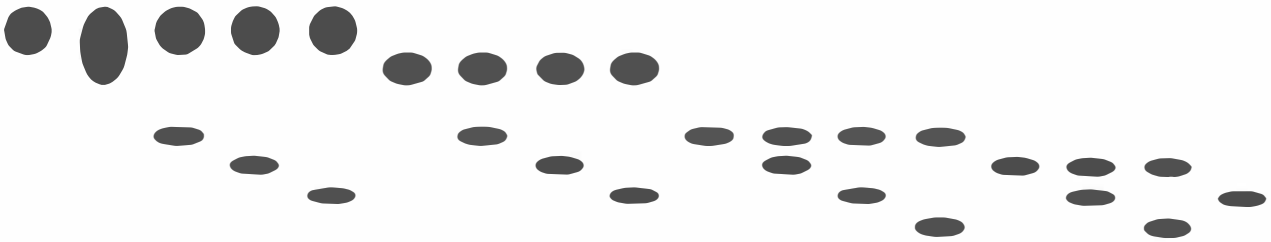
Esterase (Est) EC 3.1.1.1

Est was examined in extracts of liver tissue, and migrates anodally in Cam pH 6.1 buffer.

Subunit structure: monomeric

Observed banding pattern: one common (dd) and five other alleles were detected. Activity was good, as was the resolution of the bands, except for the two fastest migrating alleles. Heterozygotes were detected as two bands as shown below:-

Est-2



aa ab ac ad ae bb bc bd be cc cd ce cf dd de df ee

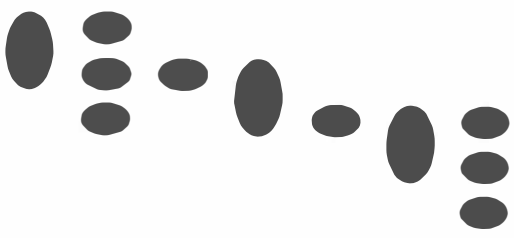
Glucosephosphate isomerase (Gpi) EC 5.3.1.9

Gpi was examined in extracts of muscle tissue. Two polymorphic loci migrate anodally in TCpH 5.8 buffer.

Subunit structure:- dimeric

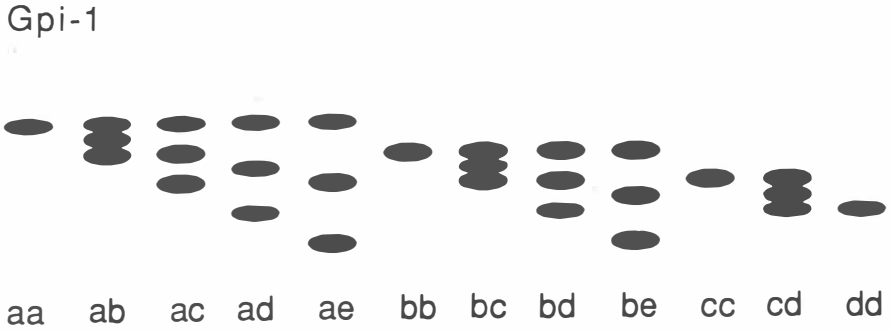
Observed banding pattern: Slow locus- one common (cc) and four rare alleles were detected. Activity was strong, with a tendancy to overstain. Heterozygotes were detected as either three bands of activity (ac, ce) or a diffuse band (bc, cd), depending on the separation of the alleles, as shown below:-

Gpi-2



ab ac bb bc cc cd ce

Fast locus- one common (aa) and four other alleles were detected. Activity was good, appearing after the slow locus had overstained. Heterozygotes were detected as three bands of activity, as shown below:-

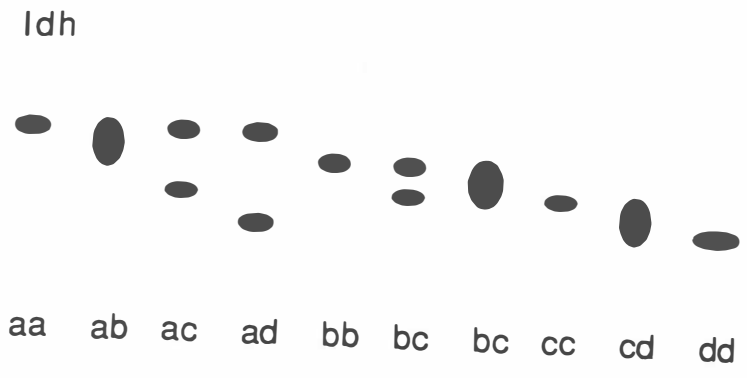


Isocitrate dehydrogenase (Idh) EC 1.1.1.42

Idh was examined in extracts of heart tissue, and migrates anodally in TC pH 6.8 buffer.

Subunit structure: dimeric

Observed banding pattern: one common (cc) and three rare alleles were detected. Activity suffered in some samples and the resolution was variable. Atypical heterozygotes were detected as two bands as shown below:-

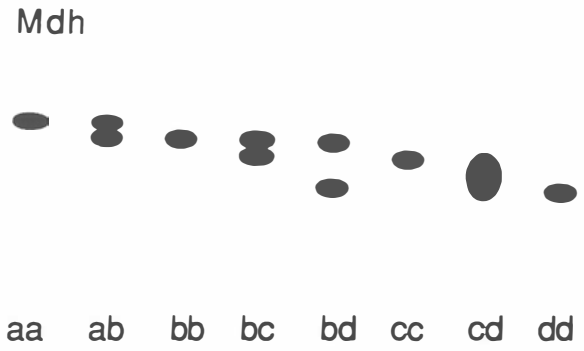


Malate dehydrogenase (Mdh) EC 1.1.1.37

Mdh was examined in extracts of heart tissue, and migrates anodally in TC pH 6.8 buffer.

Subunit structure: dimeric

Observed banding pattern: two common (bb, cc) and two other alleles were detected. Activity suffered in some samples and the resolution was variable. Atypical heterozygotes were detected as two bands as shown below:-



Malic enzyme (Me) EC 1.1.1.40

Me was examined in extracts of liver tissue, and migrates anodally in Cam pH 6.1 buffer.

Subunit structure: tetrameric

Observed banding pattern: two alleles were detected with the resolution being variable. Atypical heterozygotes were detected as either two bands or a difuse area of activity, as shown below:-

Me

aa ab ab bb

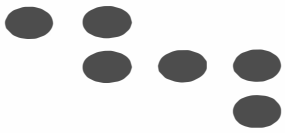
Mannosephosphate isomerase (Mpi) EC 5.3.1.8

Mpi was examined in extracts of heart tissue, and migrates anodally in TC pH 6.8 buffer.

Subunit structure: monomeric

Observed banding pattern: one common (bb) and two rare alleles were detected. Activity suffered in some samples, the resolution was variable, and this locus is prone to warping. Heterozygotes were detected as two bands, as shown below:-

Mpi



aa ab bb bc

Phosphoglucomutase (Pgm) EC 5.4.2.2

Pgm was examined in extracts of muscle tissue, and migrates anodally in TC pH 5.8 buffer.

Subunit structure: monomeric

Observed banding pattern: one common (bb) and two rare alleles were detected. Activity suffered in some samples, however the resolution was good. Heterozygotes were detected as two bands, as shown below:-

Pgm

aa ab bb bc

APPENDIX 3 Allele frequencies, heterozygosities and G-test results for polymorphic loci used in the analysis of orange roughy population structure.

Table 3.1 Allele frequencies, numbers of fish successfully scored, heterosygosity values, and goodness-of-fit tests (Chi-squared for heterozygosities, G-statistic for Hardy-Weinberg) for samples of orange roughy from the Australian collections.

Aat

OTU	p	q	r	N	HL	HL(obs)	Chi Sq	G-stat
NewZealand	0.0625	0.9375	0.0000	48	0.1172	0.1250	0.4252	0.385
EastTasman	0.1337	0.8663	0.0000	86	0.2316	0.1744	10.4916*	4.138*
WestTasman	0.0764	0.8958	0.0000	72	0.1902	0.2083	1.9561	1.456
KangIsSA	0.0455	0.9220	0.0325	77	0.1468	0.1299	2.0410	0.543
PortlandV	0.1143	0.8857	0.0000	70	0.2025	0.1714	3.3022	1.362
NSWTotal	0.1186	0.8814	0.0000	118	0.2091	0.1864	2.7814	1.190
NSWLarge	0.0250	0.9750	0.0000	40	0.0488	0.0500	0.0484	0.041
NSWSmall	0.1667	0.8333	0.0000	69	0.2779	0.2464	1.7731	0.798
NSWSmall-1	0.2097	0.7903	0.0000	31	0.3315	0.3548	0.3063	0.190
NSWSmall-2	0.1912	0.8088	0.0000	34	0.3093	0.0294	55.6871*	4.947*

Est

OTU	p	q	r	s	t	u	N	HL	HL(obs)	Chi Sq	G-stat
NewZealand	0.1200	0.0300	0.1700	0.6400	0.0200	0.0200	50	0.5454	0.4200	13.2161	3.454
EastTasman	0.0969	0.0867	0.1837	0.5714	0.0612	0.0001	98	0.6191	0.5102	15.1610	4.932
WestTasman	0.0806	0.0591	0.2312	0.6129	0.0161	0.0001	93	0.5607	0.6344	8.8339	5.645
KangIsSA	0.1234	0.1235	0.3086	0.4136	0.0309	0.0000	81	0.7022	0.8889	28.6300*	12.164*
PortlandV	0.1143	0.0786	0.2071	0.5786	0.0214	0.0000	70	0.6026	0.5286	5.2780	2.421
NSWTotal	0.1377	0.0509	0.2186	0.5599	0.0150	0.0179	167	0.6166	0.6861	10.6084	16.189*
NSWLarge	0.1228	0.0263	0.2456	0.5965	0.0088	0.0000	57	0.5680	0.5439	0.5131	3.145
NSWSmall	0.1337	0.0594	0.2178	0.5545	0.0099	0.0247	101	0.6230	0.5446	7.9574	6.736
NSWSmall-1	0.1121	0.0517	0.2414	0.5517	0.0086	0.0345	58	0.6208	0.4138	49.6570*	12.667*
NSWSmall-2	0.1628	0.0698	0.1860	0.5581	0.0116	0.0116	43	0.6223	0.7209	5.3975	8.085*

Gpi-1

OTU	p	q	r	s	t	N	HL	HL(obs)	Chi Sq	G-stat
NewZealand	0.5000	0.2041	0.2857	0.0102	0.0000	49	0.6266	0.5918	0.6046	1.479
EastTasman	0.4898	0.2347	0.2500	0.0255	0.0000	98	0.6419	0.6020	1.5146	0.702
WestTasman	0.4840	0.3138	0.1809	0.0213	0.0000	94	0.6341	0.6809	2.0482	3.088
KangIsSA	0.4835	0.2912	0.2198	0.0055	0.0000	91	0.6331	0.6593	0.6234	3.088
PortlandV	0.5074	0.2059	0.2132	0.0735	0.0000	68	0.6493	0.7647	8.5919	16.070*
NSWTotal	0.4820	0.2665	0.2365	0.0090	0.0060	167	0.6406	0.5868	4.7116	2.525
NSWLarge	0.5081	0.2742	0.1935	0.0081	0.0161	62	0.6289	0.5806	1.4628	2.200
NSWSmall	0.4316	0.2947	0.2632	0.0105	0.0000	95	0.6575	0.5789	5.4305	3.831
NSWSmall-1	0.4000	0.3182	0.2636	0.0182	0.0000	55	0.6689	0.5273	9.8589	7.668
NSWSmall-2	0.4750	0.2625	0.2625	0.0000	0.0000	40	0.6366	0.6500	0.0709	1.355

Key: HL = calculated heterozygosity HL(obs) = observed heterozygosity

* = significant at P>0.05

Table . 3.1 cont...

Gpi-2

OTU	p	q	r	s	t	N	HL	HL(obs)	Chi Sq
NewZealand	0.0204	0.0102	0.9388	0.0204	0.0102	49	0.1176	0.1224	0.3265
EastTasman	0.0101	0.0253	0.9545	0.0000	0.0101	99	0.0881	0.0909	0.4000
WestTasman	0.0160	0.0053	0.9628	0.0053	0.0106	94	0.0726	0.0638	5.5243
KangIsSA	0.0160	0.0585	0.9149	0.0106	0.0005	94	0.1591	0.1596	0.0037
PortlandV	0.0000	0.0214	0.9643	0.0143	0.0000	70	0.0695	0.0714	0.2093
NSWTotal	0.0116	0.0116	0.9480	0.0144	0.0144	173	0.1006	0.0983	0.3617
NSWLarge	0.0242	0.0323	0.9274	0.0081	0.0080	62	0.1382	0.1290	1.0990
NSWSmall	0.0049	0.0000	0.9703	0.0099	0.0149	101	0.0582	0.0594	0.1718
NSWSmall-1	0.0000	0.0000	0.9741	0.0172	0.0086	58	0.0508	0.0517	0.0728
NSWSmall-2	0.0116	0.0000	0.9651	0.0000	0.0233	43	0.0679	0.0698	0.1347

Idh

OTU	p	q	r	s	N	HL	HL(obs)	Chi Sq	G-stat
NewZealand	0.0729	0.1562	0.7292	0.0417	48	0.4368	0.4375	0.0004	1.001
EastTasman	0.0253	0.0707	0.8990	0.0050	99	0.1861	0.1212	36.1204*	5.017*
WestTasman	0.0161	0.0376	0.9462	0.0001	93	0.1030	0.0538	63.6590*	13.122*
KangIsSA	0.0115	0.2126	0.7759	0.0000	87	0.3526	0.3333	0.7820	0.345
PortlandV	0.0224	0.1642	0.8134	0.0000	67	0.3109	0.2537	6.8037	1.000
NSWTotal	0.0562	0.1331	0.8107	0.0000	169	0.3219	0.2308	40.6072*	7.285*
NSWLarge	0.0833	0.2167	0.7000	0.0000	60	0.4561	0.2833	25.8369*	10.019*
NSWSmall	0.0417	0.0937	0.8646	0.0000	96	0.2419	0.2083	5.5565	1.025
NSWSmall-1	0.0175	0.0614	0.9211	0.0000	57	0.1475	0.1229	4.7564	1.037
NSWSmall-2	0.0769	0.1410	0.7821	0.0000	39	0.3625	0.3333	0.7592	0.019

Mdh

OTU	p	q	r	s	N	HL	HL(obs)	Chi Sq	G-stat
NewZealand	0.1939	0.6020	0.0204	0.1735	49	0.5695	0.6122	0.8264	9.871
EastTasman	0.1111	0.5602	0.2172	0.1111	99	0.6139	0.5657	1.8309	28.428*
WestTasman	0.1346	0.5897	0.1667	0.1090	78	0.5945	0.5769	0.2051	22.136*
KangIsSA	0.0389	0.4722	0.2889	0.2000	90	0.6521	0.6222	0.5676	24.211*
PortlandV	0.0714	0.5714	0.1786	0.1786	70	0.6046	0.6571	1.5834	23.138*
NSWTotal	0.1107	0.5638	0.1544	0.1711	149	0.6168	0.6309	0.2336	40.281*
NSWLarge	0.1333	0.5500	0.1667	0.1500	60	0.6294	0.6167	0.0733	25.982*
NSWSmall	0.1104	0.5909	0.1429	0.1558	77	0.5940	0.6234	0.5659	13.253*
NSWSmall-1	0.1163	0.5814	0.1628	0.1395	43	0.6025	0.6279	0.2293	13.483*
NSWSmall-2	0.1029	0.6029	0.1176	0.1765	34	0.5809	0.6176	0.4071	7.418

Key: **HL** = calculated heterozygosity **HL(obs)** = observed heterozygosity

* = significant at P>0.05

Table 3.1 cont...

Me

OTU	p	q	N	HL	HL(obs)	Chi Sq	G-stat
NewZealand	0.6875	0.3125	48	0.5703	0.4167	3.4819	0.024
EastTasman	0.6471	0.3529	51	0.4567	0.3529	2.6345	2.596
WestTasman	0.6176	0.3824	85	0.4723	0.4118	1.3947	1.367
KangIsSA	0.2391	0.7609	69	0.3639	0.3623	0.0013	2.064
PortlandV	0.5606	0.4394	66	0.4927	0.5152	0.1376	1.164
NSWTotal	0.5645	0.4355	155	0.4917	0.4323	2.2621	2.263
NSWLarge	0.5784	0.4216	51	0.4877	0.4510	0.2888	0.287
NSWSmall	0.5585	0.4415	94	0.4932	0.4362	1.2555	1.257
NSWSmall-1	0.5833	0.4167	54	0.4861	0.4630	0.1219	0.417
NSWSmall-2	0.5250	0.4750	40	0.4988	0.4000	1.5694	1.554

Mpi

OTU	p	q	r	N	HL	HL(obs)	Chi Sq
NewZealand	0.0816	0.9184	0.0000	49	0.1499	0.1633	0.7831
EastTasman	0.2609	0.7337	0.0054	92	0.3936	0.2935	11.9007*
WestTasman	0.3900	0.6100	0.0000	50	0.4758	0.5000	0.2587
KangIsSA	0.2048	0.7952	0.0000	83	0.3257	0.3373	0.2106
PortlandV	0.2923	0.7077	0.0000	65	0.4137	0.4308	0.2221
NSWTotal	0.1917	0.7958	0.0125	120	0.3298	0.3083	1.0200
NSWLarge	0.2373	0.7542	0.0085	59	0.3748	0.3220	2.3418
NSWSmall	0.1296	0.8519	0.0185	54	0.2571	0.2593	0.0079
NSWSmall-1	0.1739	0.7826	0.0435	23	0.3554	0.3478	0.0210
NSWSmall-2	0.0968	0.9032	0.0000	31	0.1749	0.1935	0.7012

Pgm

OTU	p	q	r	N	HL	HL(obs)	Chi Sq
NewZealand	0.0918	0.8980	0.0102	49	0.1851	0.2041	1.0326
EastTasman	0.1990	0.8010	0.0000	98	0.3188	0.2551	7.8253*
WestTasman	0.1437	0.8563	0.0000	87	0.2461	0.2184	2.2044
KangIsSA	0.1461	0.8315	0.0224	89	0.2868	0.2697	0.6328
PortlandV	0.0909	0.9015	0.0176	66	0.1614	0.1970	6.4219
NSWTotal	0.0949	0.9051	0.0000	137	0.1718	0.1752	0.1073
NSWLarge	0.0968	0.9032	0.0000	62	0.1749	0.1935	1.4024
NSWSmall	0.0870	0.9130	0.0000	69	0.1589	0.1739	1.2297
NSWSmall-1	0.0513	0.9487	0.0000	39	0.0973	0.1026	0.2314
NSWSmall-2	0.1167	0.8833	0.0000	30	0.2063	0.2333	1.0277

Key: HL = calculated heterozygosity HL(obs) = observed heterozygosity

* = significant at P>0.05

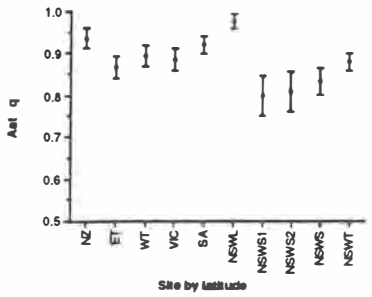
Table 3.2 G-Tests for samples of orange roughy from the Australian collections.

OTU 1	VS	OTU 2	G STAT	D OF	F	PROB.
NewZealand		EastTasman	106.4729	32		.0000 *
		WestTasman	116.4756	32		.0000 *
		KangIsSA	156.1675	31		.0000 *
		PortlandV	76.6226	31		.0000 *
		NSWTotal	69.6343	33		.0002 *
		NSWLarge	59.6861	33		.0030 *
		NSWSmall	86.4259	32		.0000 *
		NSWSmall-1	97.3787	32		.0000 *
		NSWSmall-2	42.9997	32		.0926
EastTasman		WestTasman	84.2481	32		.0000 *
		KangIsSA	120.9183	32		.0000 *
		PortlandV	62.8511	32		.0009 *
		NSWTotal	106.1205	32		.0000 *
		NSWLarge	79.5882	32		.0000 *
		NSWSmall	103.8868	31		.0000 *
		NSWSmall-1	115.5363	31		.0000 *
		NSWSmall-2	73.6301	31		.0000 *
WestTasman		KangIsSA	176.8796	32		.0000 *
		PortlandV	72.9380	32		.0001 *
		NSWTotal	79.3793	33		.0000 *
		NSWLarge	85.4308	33		.0000 *
		NSWSmall	54.6138	32		.0077 *
		NSWSmall-1	55.0923	32		.0068 *
		NSWSmall-2	63.6847	31		.0005 *
KangIsSA		PortlandV	77.5164	28		.0000 *
		NSWTotal	139.3889	33		.0000 *
		NSWLarge	85.4541	31		.0000 *
		NSWSmall	153.2559	31		.0000 *
		NSWSmall-1	158.6544	31		.0000 *
		NSWSmall-2	93.6441	31		.0000 *
PortlandV		NSWTotal	58.0768	33		.0045 *
		NSWLarge	53.4431	31		.0074 *
		NSWSmall	61.0863	31		.0010 *
		NSWSmall-1	81.8194	30		.0000 *
		NSWSmall-2	51.9052	31		.0108
NSWTotal		NSWLarge	38.9009	32		.1866
		NSWSmall	19.3495	32		.9616
		NSWSmall-1	42.5956	32		.0996
		NSWSmall-2	23.6655	33		.8842
NSWLarge		NSWSmall	71.9952	31		.0000 *
		NSWSmall-1	93.2335	31		.0000 *
		NSWSmall-2	51.1646	32		.0172
NSWSmall-1		NSWSmall-2	42.8736	30		.0600

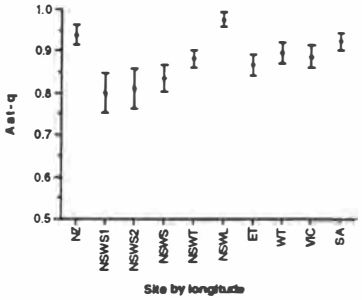
Key: * = significant at P>0.01

Appendix 4 Allele frequencies of polymorphic loci presented by latitude and longitude scattergrams.

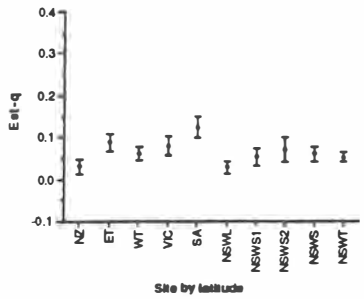
4.1a



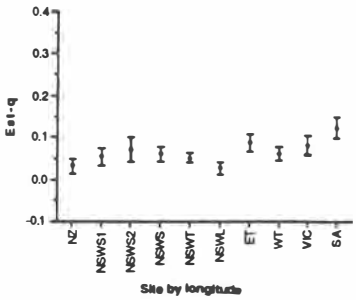
4.1b



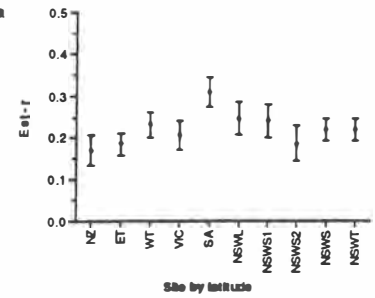
4.2a



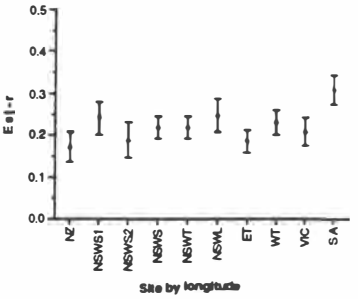
4.2b



4.3a



4.3b



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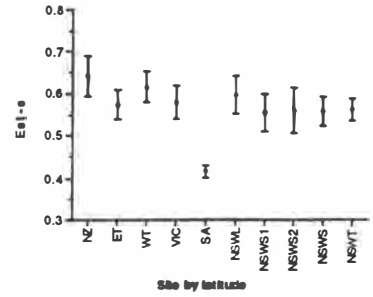

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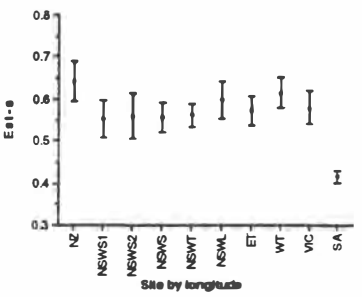
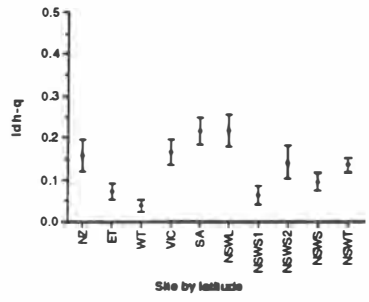


4.5a



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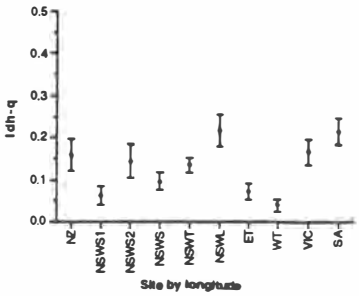
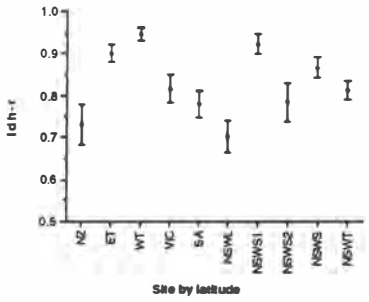


4.6a



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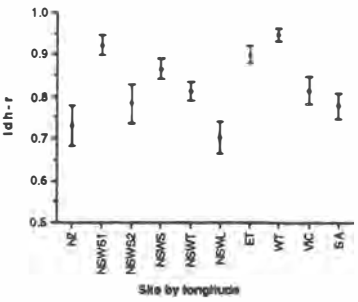

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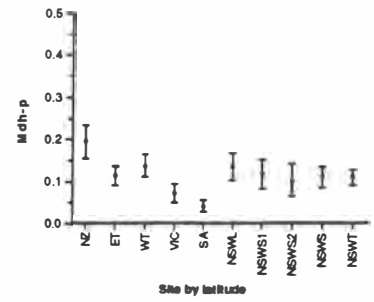

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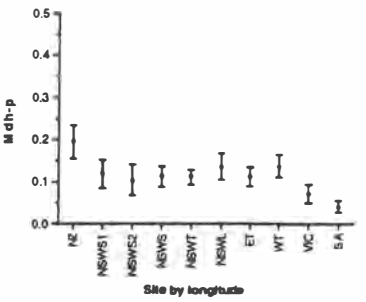

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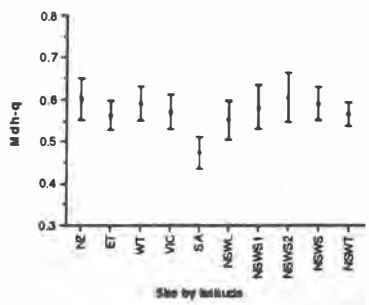

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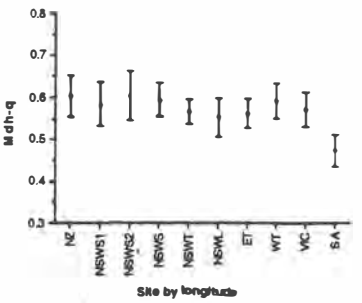

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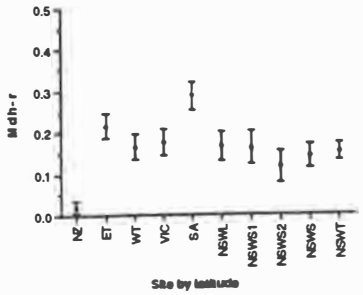
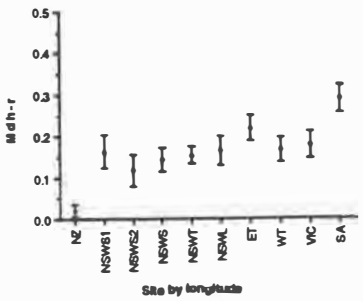


4.9b



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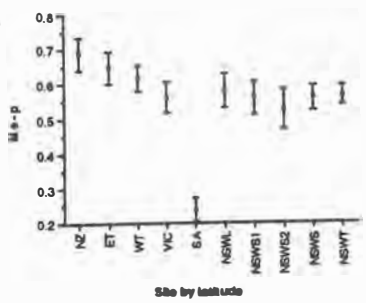

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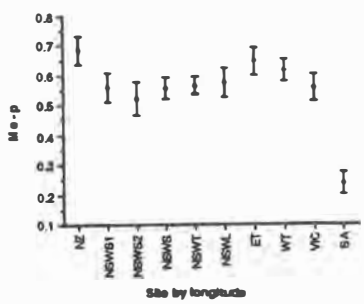

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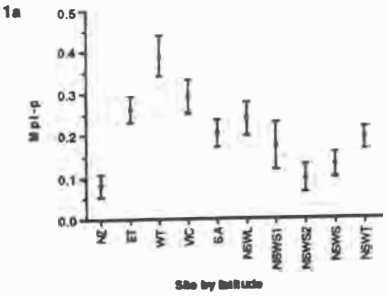

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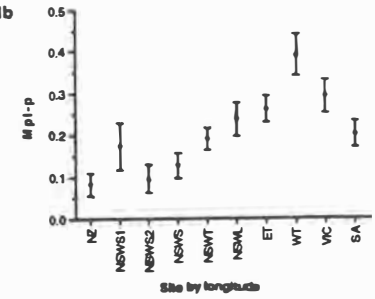
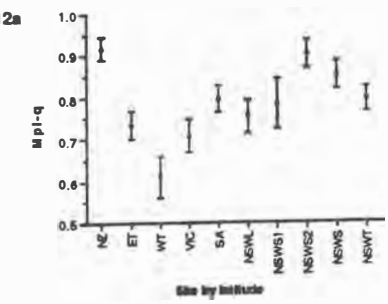
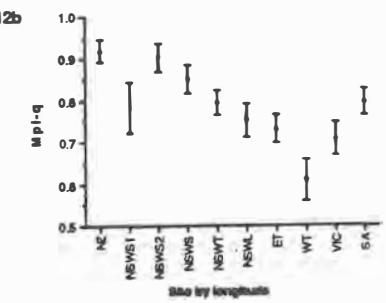


4.12a



4.12b



Appendix 5 G-tests and dendrogram (CONTML plot) for the polymorphic loci published by Smith (1986) for the analysis of orange roughy population structure in New Zealand.

Smith (1986) conducted an electrophoretic study on orange roughy from New Zealand and one locality in the Atlantic ocean. Here, we analyse these data further.

Smith's samples were analysed by pairwise comparison, using the G-statistic, to determine how orange roughy may be geographically separated around New Zealand. Significant differences were found between 13 out of 15 comparisons (see Table 5.1). Only the two samples from close to the eastern shore of New Zealand (Wairapara coast off the North Island, and Kaikoura coast off the South Island), were not significantly different.

Table 5.1 G-test results for samples of orange roughy from New Zealand (analysis of data from Smith, 1986)

OTU 1 VS	OTU 2	G STAT	D OF F	PROB.
NEAtlantic	Chatham	68.9111	39	.0022 *
	Kaikoura	58.2132	35	.0082 *
	Wairarapa	35.9237	35	.4251
	Challeng-1	65.0491	40	.0074 *
	Challeng-2	61.3671	37	.0072 *
Chatham	Kaikoura	63.1291	37	.0048 *
	Wairarapa	82.5447	39	.0001 *
	Challeng-1	88.1966	40	.0000 *
	Challeng-2	120.2170	40	.0000 *
Kaikoura	Wairarapa	52.9833	35	.0262
	Challeng-1	62.4919	38	.0074 *
	Challeng-2	116.1414	38	.0000 *
Wairarapa	Challeng-1	83.6043	40	.0001 *
	Challeng-2	71.6477	39	.0011 *
Challeng-1	Challeng-2	132.6024	41	.0000 *

Smith's allele frequency data was also used to construct a dendrogram to show the genetic relationships between the localities sampled. We used Felsenstein's (1981,1982) CONTML program to construct the maximum likelihood phylogenetic tree. The dendrogram widely separates the sample from NE Atlantic ocean. Within New Zealand, however, the dendrogram is not clustered in a geographic way, and the two samples that were not significantly different by G-test are separated in the dendrogram. (Refer to Figure 5.1).

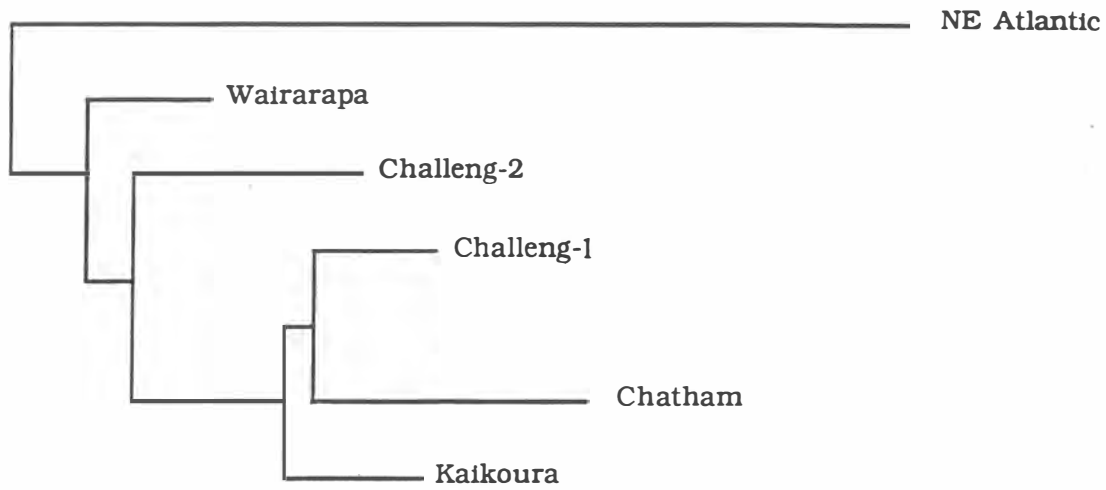
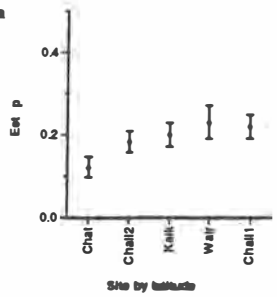


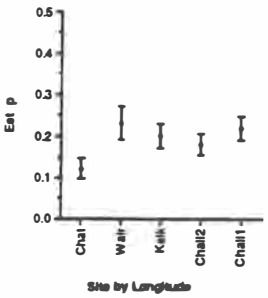
Figure5.1 Dendrogram to show the relationships between orange roughy samples from New Zealand (analysis of data from Smith, 1986). (CONTML plot).

To examine the relationships of orange roughy around New Zealand further, allele frequencies are presented by latitude and longitude as scattergrams in Figures 5.2-5.9, below.

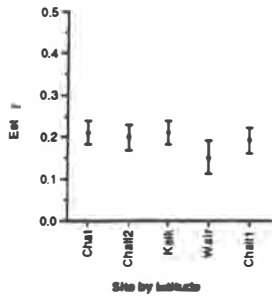
5.2a



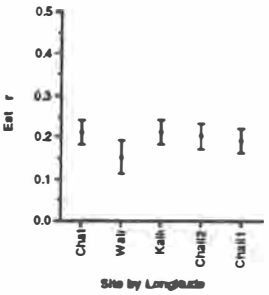
5.2b



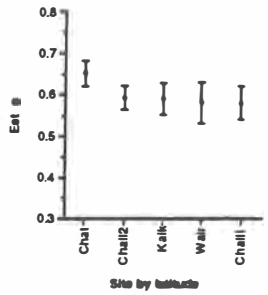
5.3a



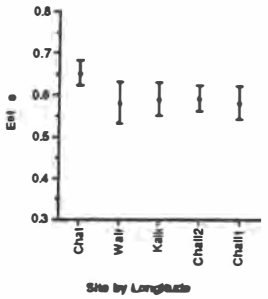
5.3b



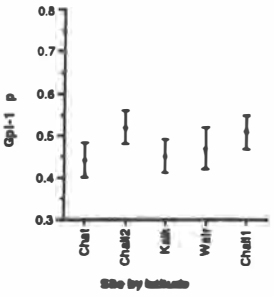
5.4a



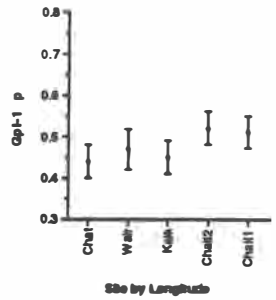
5.4b



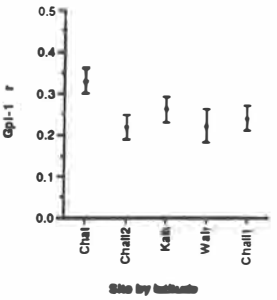
5.5a



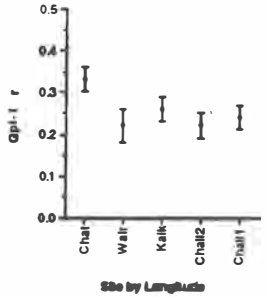
5.5b

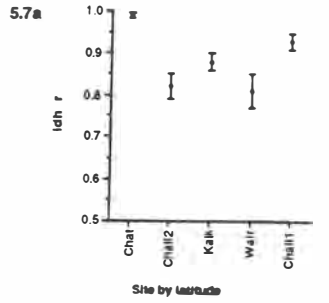


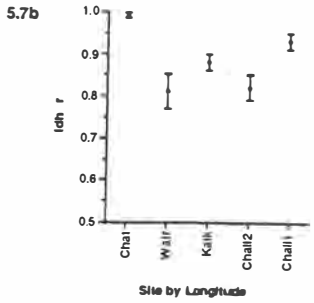
5.6a

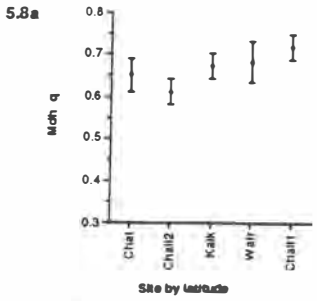


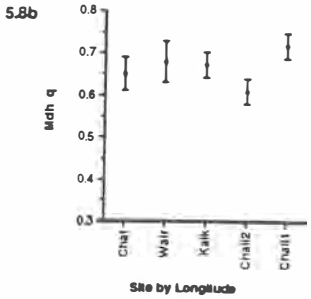
5.6b

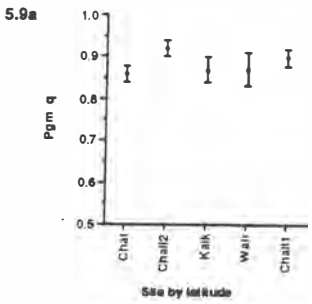


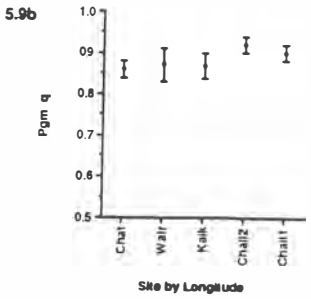












Chatham Rise is separated by:- Est^{p} (Figure 5.2a&b), Est^{s} (Figure 5.4a&b), Gpi-1^{r} (Figure 5.6a&b), Idh^{r} (Figure 57a&b),and Mdh^{q} (Figure 5.8a). Samples taken from the west coast, Challenger Plateau, separate from the other localities at:- Gpi-1^{p} (Figure 5.5b), and Idh^{r} (Figure 5.8b). The Wairarapa coast, from the North Is., is separated by:- Est^{r} (Figure 5.3a&b) and Idh^{r} (Figure 5.7a&b).