Population structure of the blue-eye (deepsea trevalla), *Hyperoglyphe antarctica*

Project 1991/19
Fishing Industry Research and Development Trust Fund

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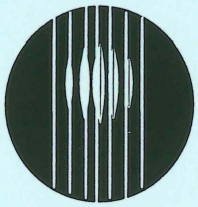

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Final Report

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Project 1991/19

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CONTENTS

1. Non-technical summary

1. The "big eye" and "small eye" morphs represent developmental stages of the blue-eye and not separate species.

An analysis of relative eye size (eye size divided by standard length) shows that within the size range 400-500 mm, relative eye size increases sharply, while in fish greater than 500 mm the rate of increase in eye size declines. Variation in eye size appeared to reflect ontogenetic changes within the species rather than variation between species. The "small eye" and "big eye" forms represent two life history stages, with metamorphosis between the two taking place at about 400-500 mm standard length. This conclusion is supported by meristic and genetic analyses of fish classified as "small eye" and "big eye". Not one of six meristic characters showed statistically significant differences between the two morphs. A genetic analysis of 37 loci also failed to reveal significant differences at any locus, strongly suggesting that the forms represent different morphs within a species rather than separate species.

3. A previously unrecognised species of trevalla, the "ocean blue-eye" (provisionally identified as Schedophilus labyrinthicus) was discovered off New South Wales.

Three out of 96 heads sent from the Taupo and Barcoo seamounts for the stock structure study were observed to have different shapes. These three specimens were also highly different genetically from other blue-eye that had been examined. Subsequent examination of some whole specimens of this unusual form showed that they had different dorsal and anal fin ray counts from blue-eye. This second blue-eye species has been provisionally named "ocean blue-eye", and its distribution overlaps with the common blue-eye off New South Wales.

2. There is no evidence for genetic stock structure of blue-eye trevalla in the southeast trawl region.

Six samples (n=67 to 154) of blue-eye or deepsea trevalla were collected from south eastern Australia (seamounts off New South Wales, a seamount south east of Tasmania called the Cascade Plateau, off the east, south and west coasts of Tasmania,

and off the coast of South Australia). All fish were analysed by starch or cellulose acetate electrophoresis for the products of seven variable loci, and a minimum of 24 fish per area were analysed for 29 other less variable loci. Several statistical analyses of the data were carried out: none revealed any significant inter-sample differentiation for any locus. These results indicate that gene flow is sufficient to prevent any genetic differentiation among the sampled localities.

2. Background

The blue-eye (*Hyperoglyphe antarctica*) belongs to the cosmopolitan fish family Centrolophidae, known collectively as trevallas. The genus *Hyperoglyphe* contains 6 valid species with three occurring in the Southern Hemisphere (Haedrich and Horn, 1972). Only one species is thought to occur in Australasian waters although more than one form has been recognised within the fishery. The Australian blue-eye is thought to be widespread in the Southern Ocean, occurring in continental shelf and upper slope waters off the coasts of southern Australia, New Zealand, South Africa, Tristan da Cunha, in the southern Indian Ocean and off southern South America (Haedrich, 1967; McDowall, 1982; Paul, 1986).

Cowper and Downie (1957) were first to establish that commercially significant quantities of blue-eye existed in Australian waters. Their work off the east coast of Tasmania was followed by a further surveys which confirmed the existence of exploitable stocks in other regions, such as off western Tasmania (Wilson, 1981), Victoria (Winstanley, 1979) and South Australia (Jones, 1985). It has since become clear that within the Australian Fishing Zone, blue-eye are found from mid-New South Wales to southwestern West Australia, on the outer continental shelf and upper slope, and around seamounts such as the Tasman Rise, Cascade Plateau, and those off New South Wales. Despite this knowledge, nothing is known of stock structure within the fishery.

The blue-eye resource has been exploited primarily through the use of droplines in the 100-600 m depth zone. This fishery currently lands about 800 tonnes annually with a market value in excess of $3.6 million (H. Williams, Tasmanian Division of Sea Fisheries, pers. comm.). It is the premium marine table fish of Tasmania and is also a high value species in New South Wales and Victoria. Sashimi quality blue-eye have recently been exported to Japan from New South Wales.

In recent years, as orange roughy (*Hoplostethus atlanticus*) grounds became subject to temporary closures and catch restrictions on roughy were imposed in an effort to protect what were judged to be

dwindling stocks, some fishermen diversified to mid-water trawling. Such fishing methods had the potential of greatly increasing trawl catches of blue-eye, either from direct targeting or as a bycatch from targeting other species such as blue grenadier (*Macruronus novaezelandiae*). Indeed, trawler catches of blue-eye have increased from around 30 tonnes annually in 1986 and 1987 to around 200 tonnes in 1991. Analyses of CPUE statistics in New Zealand suggested a decline in the abundance of blue-eye in heavily trawled areas since the development of a blue-eye/alfonsino mid-water trawl fishery in 1983 (Horn and Massey, 1989). Australian drop-line fishermen were concerned that mid-water trawlers could deplete blue-eye stocks very rapidly and threaten their traditional fishery with extinction. Their complaints led to the introduction of a 500 kg trip limit for trawlers in early 1991. A TAC imposed in 1992 allowed the removal of this trip-limit with the aim of minimising targeted trawling for blue-eye.

Little is known of the biology of blue-eye. The egg and larval stages remain undiscovered and, until recently, little was known of the juvenile stages. Fish of less than 45 cm snout to caudal fork length (LCF) are rarely caught by commercial vessels. Horn (1988) and Horn and Massey (1989) assumed that, like other stromateoid fishes, including some other species of *Hyperoglyphe* (Haedrich, 1967; Dawson, 1971), juveniles were surface-living. This supposition has been confirmed recently following the discovery of juvenile blue-eye amongst floating kelp rafts (Last et al., 1993, see Appendix 1). Two AFZ observers on Japanese longliners, Lisa Hick and Ian Peel, collected the first juvenile blue-eyes (ranging in size from about 30–60 mm) from Australasian waters. These specimens were collected in June/July amongst floating rafts of the brown kelp, *Phyllospora comosa,* from beyond the shelf break off eastern Tasmania. The location of fish between 6–42 cm is still unknown, although blue-eye smaller than 58 cm sometimes aggregate near the surface where they can be caught by trolling and midwater trawling.

Studies in southern Australian and New Zealand waters indicate that individuals grow very rapidly in the first few years of life, reaching LCF's ≤ 30 cm in their first year and ≤ 50–60 cm in their second year; followed by a reduction in the rate of growth thereafter (Webb, 1979; Jones, 1985; Horn, 1988).

The adults (>47 cm LCF) are bathypelagic/demersal, and are predominantly found over rocky bottoms on the shelf break and upper continental slope in depths of 200–900 m (Webb, 1979; Jones, 1988). Recruitment into both Australian and New Zealand commercial fisheries occurs at between two and three years of age, with estimates of the age at sexual maturity varying from three to five years (Horn and Massey, 1989) to six to seven years (Webb, 1979). Most of the commercial catch consists of immature specimens (Webb, 1979; Horn, 1988). Jones (1988) found South Australian fish to spawn in April, whereas Webb (1989) found Tasmanian fish spawning in summer. Baelde (pers. com.) observed mature fish off the east coast of Tasmania between January and March which coincides well with the regional occurrence of small juveniles in winter. New Zealand fish are thought to spawn in late summer (Horn and Massey, 1989). Blue-eye are believed to live to around 13 to 15 years (Jones, 1985; Horn, 1988), although ageing methods have not yet been validated. Maximum recorded sizes range from 90 cm from Victoria (Winstanley and Smith, 1982), 99 cm from South Australia (Jones, 1985), 107 cm from the Cascade Plateau (Williams, 1989) to 137 cm from New Zealand (McDowall, 1982).

Adult blue-eye aggregate on the bottom at night and rise into the water column during the day to feed (Winstanley, 1978). Apart from these diel movements, adults are generally thought to be residential for most of the year, although the species is capable of long and fairly rapid migrations. Short term tagging studies carried out off New Zealand showed that 36 out of 40 tagged and recaptured specimens were caught on the grounds on which they were tagged, although one of the other fish moved 490 km in 137 days and a second fish moved 450 km in 231 days (Horn, 1989).

Little is known concerning the resource status of blue-eye. Wankowski and Moulton (1986) estimated the blue-eye biomass for Eastern Bass Strait at only 800 tonnes. This figure was extrapolated from demersal trawl surveys and is likely to be an underestimate because blue-eye typically occupy rough untrawlable ground. Williams (1993) concluded that the resource was almost fully exploited, and in some areas there is evidence of localised depletion indicating possible over-exploitation.

In summary, poor biological resource information for the blue-eye fishery makes it difficult to devise an effective and efficient management policy. For example, there are no good biomass estimates, no sustainable yield estimates, and, prior to the commencement of this project, no information on stock structure. Although at least two distinct morphotypes (distinguishable on eye size, body shape and colour) exist within the commercial catch, the species composition of the fishery had never been investigated. A knowledge of species composition and stock structure are basic pieces of information needed to manage a fishery. This study investigated these aspects of the Australian blue-eye fishery.

3. Project details

3.1 Objectives

* To investigate species composition of blue-eye in southern Australian waters through a detailed morphological and genetic comparisons of two identifiable morphs, and, if two species do exist, to prepare guideline information on their spatial and geographic distributions.

* To assess stock structure and to determine the approximate extent of gene flow among populations taken from different areas of southern Australia.

3.2 Personnel

Christopher Bolch was appointed to this one year project and carried out most of the genetic and morphological analyses. Training in genetic techniques was provided by Nick Elliott and Bob Ward, and in classical taxonomy and morphometrics by Peter Last. Project supervisors were Bob Ward and Peter Last.

4. Species composition of the blue-eye fishery

4.1 Introduction

The trevalla genus *Hyperoglyphe* Gunther presently contains six species (Haedrich, 1967; Haedrich and Horn, 1972). Of these, three have been recorded from the Southern Hemisphere: *H. antarctica* (Carmichael), *H. moselii* (Cunningham) and *H. macrophthalma* (Miranda-Ribeiro). The blue-eye, *H. antarctica*, is widespread in temperate waters with its range including all southern continents, many islands and some seamounts. The other two species are confined to the Atlantic Ocean although *H. moselii* occurs together with *H. antarctica* off South Africa.

Recently, a second form of blue-eye was recognised by fishermen and scientists in Australian waters. This morph is paler, deeper bodied, has a more evenly rounded head, and proportionally smaller eye than the 'typical' blue-eye. The iris of its eye is also yellowish rather than bluish. The taxonomic relationships of these forms had not been investigated and at the start of this project it was uncertain whether these morphs were distinct species or simply morphs of *Hyperoglyphe antarctica.* If these forms are of one species, do they constitute growth phases or are they normal shape and colour variants within a variable species? If these morphs are valid species, the scientific name of the blue-eye may be incorrect and catch data collected to manage the fishery may be invalid. Interestingly, other scientific names have been applied in the past to blue-eye from Australia, although McDowall (1982), without examining material from Australia, considered these names, *Hyperoglyphe porosa* (Richardson) and *H. johnstonii* (Morton), to be junior synonyms of *H. antarctica.*

The species question and the resolution of blue-eye morphs was addressed by a combination of both classical taxonomic and genetic techniques.

4.2 Blue-eye morphs

In order to establish the taxonomic relationships of these two forms, 97 whole fish, collected from the east and west coasts of Tasmania in October and December 1991 respectively, were examined by allozyme electrophoresis. Fish were classified, subjectively, as "big eye", "small eye" or indeterminate. The genetic methods used are outlined in section 5.2.

Morphological and meristic data were taken from the genetic samples. In addition, 13 fish, which had already been gutted and therefore could not be sexed or genetically screened, were also measured although meristics were not taken. Shape measurements concentrated on estimates of body size (standard and fork lengths, taken to an accuracy of 1 mm) and eye size (including eye diameter, external eye diameter, and eye socket diameter, taken by calipers to an accuracy of 0.1 mm).

4.2.1 Genetic study of blue-eye morphs

Allele frequencies at 37 loci of the "big eye" and "small eye" groups are given in Table 4.1. There are no significant differences in allele frequency at any locus, and the genetic identity between the forms is extremely high (Nei's standard genetic identity (1972) = 0.999; Nei's unbiased genetic identity (1978b) = 1.000). The two groups of fish could not be separated genetically, indicating very strongly that the forms represent different morphs within a species rather than two distinct species. Had the two forms been distinct species, and therefore reproductively isolated from each other, a number of diagnostic loci would have been expected, together with significant differences in allele frequencies at other loci. Note that large genetic differences were observed between blue-eye and the ocean blue-eye, section 4.3.

Table 4.1. Allele frequencies of fish classified as big-eye and small-eye. Probabilities are derived from Roff and Bentzen (1989) Monte-Carlo chi-square method, using 1000 randomisations.

Locus	Allele	Allele frequencies: Big eye (n =25)	Small eye (n =59)	*P*
*sAAT-1**	115	0.220	0.237	*0.850*
	100	0.780	0.763	
*sAAT-2**	100	1.000	0.983	*0.614*
	60	-	0.017	
*mAAT**	90	-	0.008	*0.488*
	-30	0.080	0.034	
	-100	0.920	0.958	
*ACP**	100	1.000	1.000	-
*ADA**	125	0.060	-	*0.740*
	105	-	0.008	
	100	0.840	0.864	
	95	0.080	0.042	
	80	0.020	0.008	
	55	-	0.017	
*ADH**	-55	0.440	0.441	*1.000*
	-100	0.560	0.559	
*AK**	100	1.000	1.000	-
*CK-1**	100	1.000	0.983	*0.604*
	95	-	0.017	
*CK-2**	100	1.000	1.000	-
*CK-3**	100	1.000	1.000	-
*ESTD**	100	1.000	1.000	-
*FH**	100	1.000	1.000	-
*G3PDH-1**	100	1.000	1.000	-
*G3PDH-2**	100	1.000	1.000	-
*G6PDH**	100	1.000	1.000	-
*GAPDH-1**	100	1.000	1.000	-
*GAPDH-2**	100	1.000	1.000	-
*GDA**	100	1.000	1.000	-
*GPI-1**	130	0.040	0.017	*0.484*
	100	0.960	0.983	
*GPI-2**	100	1.000	1.000	-
*IDDH**	100	1.000	1.000	-
*IDH1-1**	100	1.000	1.000	-
*IDH1-2**	100	1.000	1.000	-
*LDH-1**	100	1.000	1.000	-
*LDH-2**	100	1.000	1.000	-
*LDH-3**	100	1.000	1.000	-
*sMDH-1**	100	1.000	1.000	-
*sMDH-2**	100	1.000	1.000	-
*ME1**	100	1.000	1.000	-
*MPI**	115	0.020	-	*0.212*
	100	0.940	0.983	
	85	0.040	0.017	
*ODH**	120	0.015	0.010	*1.000*
	100	0.985	0.990	
*PEP1**	100	1.000	0.992	*1.000*
	85	-	0.008	
*PEP2**	105	-	0.008	*0.654*
	100	0.900	0.822	
	90	0.100	0.161	
	85	-	0.008	
*PGDH**	115	-	0.017	*0.266*
	100	0.900	0.805	
	65	0.100	0.178	
*PGM-2**	350	0.060	0.034	*0.782*
	100	0.940	0.958	
	-150	-	0.008	
*SOD1**	100	1.000	1.000	-
*XDH**	100	1.000	1.000	-

4.2.2. Morphological studies of blue-eye morphs

Meristics

Seven meristic features are generally considered to be useful in distinguishing between trevalla species: dorsal-fin spines and rays, anal-fin spines and rays, pectoral-fin rays, vertebrae, and rakers of the outer gill arches. Three of these characters, vertebrae, and dorsal and anal-fin spines, were invariable (Table 4.2). Of the remaining characters, only one, right pectoral rays, showed a significant difference between big-eye and small-eye fish. Differences in this count were probably a statistical artifact as one would have expected left pectoral-fin rays to also show a similar difference if they were real. Pectoral-fin counts were not significantly different between forms following Bonferroni adjustment to allow for the multiple tests.

Meristic data for commercial blue-eye (Table 4.3) agree closely with data published for *H. antarctica* (McDowall, 1980) with the following variations: in our material dorsal-fin rays 18–21 (vs 15–20); anal-fin rays 13–16 (vs 14–16); pectoral fin rays 18–22 (vs 19–21); and gill rakers 23–27 (vs 22–25).

Morphometrics

The morphometrics of the head in blue-eye appear to change with age. Eye size increased with body size but the rates of increase appear to vary within different size intervals (Fig 4.1). A LOWESS smoothing curve, as produced by the SYSTAT statistics package, produces a smooth curve by running along the X values (standard length) and finding predicted values from a weighted average of nearby Y values (external eye diameter). Each running window includes half the data points. The lack of data in the region of 90 to 350 mm means that no slope is given for this region. Between about 400 and 500 mm, the slope had a steep gradient suggesting ontogenetic changes in form. However, in fish >500 mm the gradient was much less than at the previous size interval.

Relative eye size is likely to be more informative than absolute eye size in reflecting growth related

Table 4.2. Morphometrics and meristics of blue eye trevalla classified as "small eye" and "big eye". Dorsal fin spines (9, n=59, 25), anal fin spines (3, n=59, 25), trunk vertebrae (10, n=6, 6) and tail vertebrae (15, n=6, 6) were invariant.

Trait		small eye	big eye	*P*
standard length (mm)	mean	428.051	543.200	<0.001
	n	59	35	
	SD	14.014	82.764	
dorsal fin rays	mean	19.160	19.500	0.539
	n	59	24	
	SD	0.788	0.590	
anal fin rays	mean	14.695	14.609	0.485
	n	59	23	
	SD	0.500	0.499	
left pectoral rays	mean	20.508	20.320	0.166
	n	59	25	
	SD	0.569	0.557	
right pectoral rays	mean	20.542	20.160	0.018
	n	59	25	
	SD	0.625	0.746	
left gill rakers	mean	25.119	25.120	0.995
	n	59	25	
	SD	0.930	0.927	
right gill rakers	mean	25.088	25.174	0.705
	n	57	23	
	SD	0.872	1.029	
external eye diameter (mm)	mean	26.755	40.090	<0.001
	n	55	35	
	SD	2.251	5.302	
RES (relative eye size)	mean	6.239	7.412	<0.001
	n	55	35	
	SD	0.462	0.456	

Table 4.3. Summary of meristic data on blue eye.

TOTAL OBSERVATIONS: 97

	DS	DR	AS	AR	LPUBR	LPBR	RPUBR	RPBR	LGR1
N OF CASES	97	96	97	95	97	97	97	97	97
MINIMUM	9.000	18.000	3.000	13.000	1.000	17.000	2.000	15.000	6.000
MAXIMUM	9.000	21.000	3.000	16.000	3.000	20.000	3.000	20.000	9.000
MEAN	9.000	19.615	3.000	14.695	2.021	18.402	2.021	18.402	7.608
STANDARD DEV	0.000	0.731	0.000	0.507	0.203	0.623	0.143	0.717	0.531

	LGR2	LGR3	RGR1	RGR2	RGR3	LP	RP	LGR	RGR
N OF CASES	97	97	97	97	93	97	97	97	93
MINIMUM	1.000	15.000	7.000	1.000	15.000	19.000	18.000	23.000	23.000
MAXIMUM	1.000	18.000	9.000	1.000	18.000	22.000	22.000	27.000	27.000
MEAN	1.000	16.526	7.526	1.000	16.634	20.423	20.423	25.134	25.161
STANDARD DEV	0.000	0.614	0.522	0.000	0.586	0.592	0.659	0.931	0.912

Trunk vertebrae (invariant, 10, n=12), tail vertebrae (invariant, 15, n=12)

DS=dorsal fin spines, DR=dorsal fin rays,
AS=anal fin spines, AR=anal fin rays,
LPUBR=left pectoral fin unbranched rays, LPBR=left pectoral branched rays
RPUBR=right pectoral fin unbranched rays, RPBR=right pectoral branched rays,
LGR1=upper left gill rakers, LGR2=left middle arch gill rakers, LGR3=upper left gill rakers
RGR1=upper right gill rakers, RGR2=right middle arch gill rakers, RGR3=upper right gill rakers
LP=left pectoral rays (=LPUBR+LPBR)
RP=right pectoral rays (=RPUBR+RPBR)
LGR=left gill rakers (=LGR1+LGR2+LGR3)
RGR=right gill rakers (=RGR1+RGR2+RGR3)

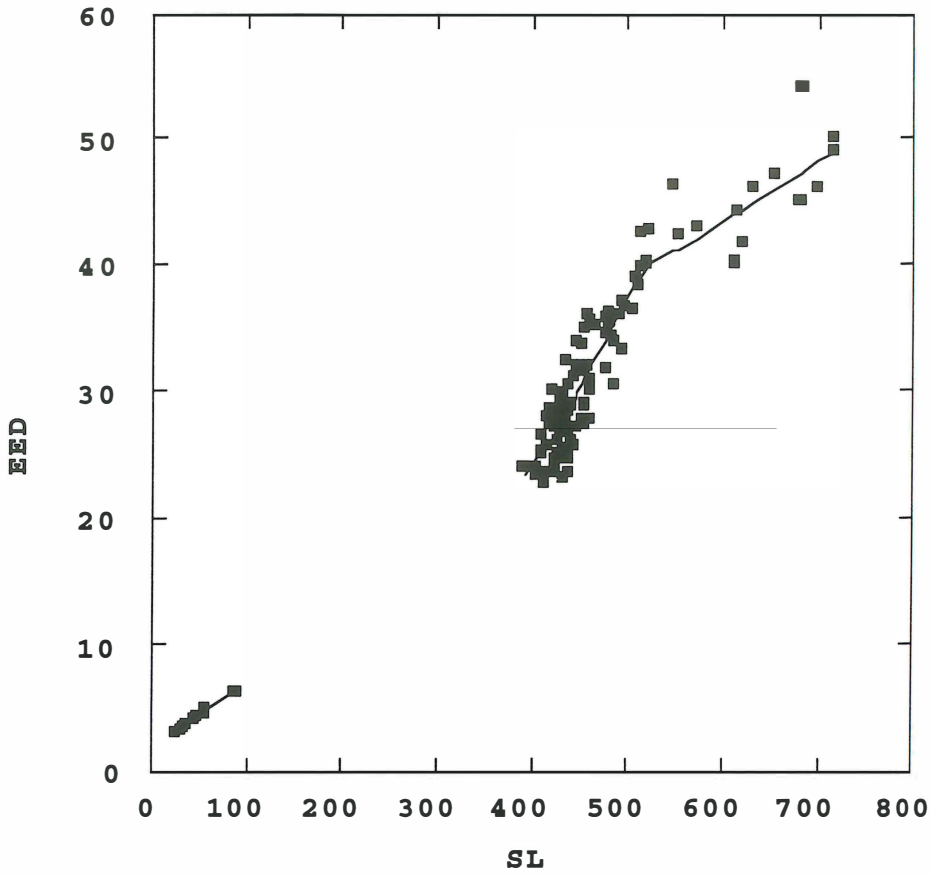

Fig. 4.1. External eye diameter (EED) plotted against standard length (SL, mm), with Lowess curves.

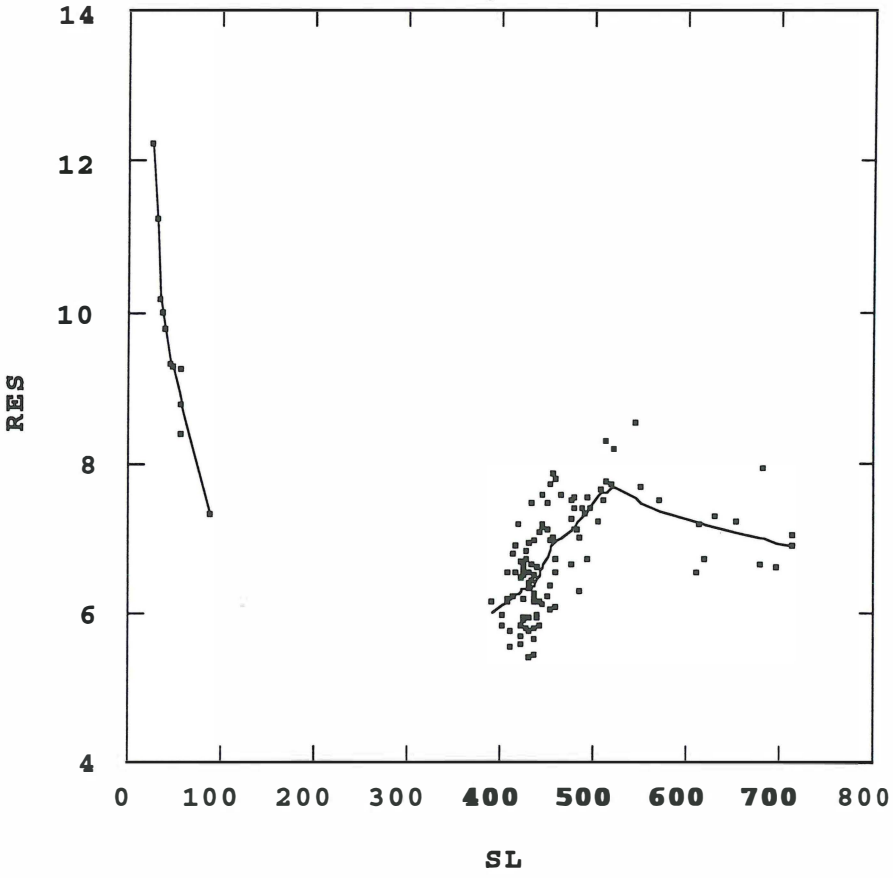

Fig.4.2. Relative eye size (RES) plotted against standard length (SL, mm), with Lowess curves.

changes in morphology. Eye size was expressed as a percentage of standard length (RES) (ie RES = 100 x (external eye diameter/standard length)) and then plotted against standard length (SL) (Fig. 4.2). The LOWESS curves show that RES declines dramatically with increasing standard length in the juvenile fish less than 90 mm.The lack of data in the region of 90 to 350 mm means that no slope is given for this region. In blue-eye recruited to the fishery, RES increases sharply as fish grow from 350 to 500 mm standard length. In the largest fish, RES appears to decline with size although data in this region are still limited.

As expected, both mean standard length and mean eye sizes were significantly greater for the those fish classified subjectively as "big-eye" than those classified as "small-eye", as was relative eye size (Table 4.2). Small-eyed fish had a mean RES of 6.24, while big-eyed fish had a mean RES of 7.41.The change in relative eye size appears to be coincidental with the onset of maturity (Fig. 4.3).

4.3. Discovery of ocean blue-eye

In the course of the stock structure study, a batch of 96 blue-eye heads and livers were sent frozen to us by the Fortuna Fishing Company. These had been removed from blue-eye caught by Mike Rowley on the Taupo and Barcoo seamounts off New South Wales. While extracting tissue samples from these carcasses, we observed that three of the heads differed in shape from the rest. These fish were flagged for future reference. Some previously unobserved genotypes were recorded from genetic analyses of these unusual specimens. These genotypes were sometimes homozygous for alleles not previously observed even in the heterozygous condition, a finding indicative of strong reproductive isolation.

These findings were discussed with Kevin Rowling (NSW Fisheries Research Institute) who intimated that he had previously suspected the existence of a second trevalla species off New South Wales, and believed that significant catches of this second species were sometimes made. These fish were sold with typical blue-eye in the Sydney fish market. He was able to collect two gutted specimens of the second species for closer morphological and genetic examination. Unfortunately their livers were

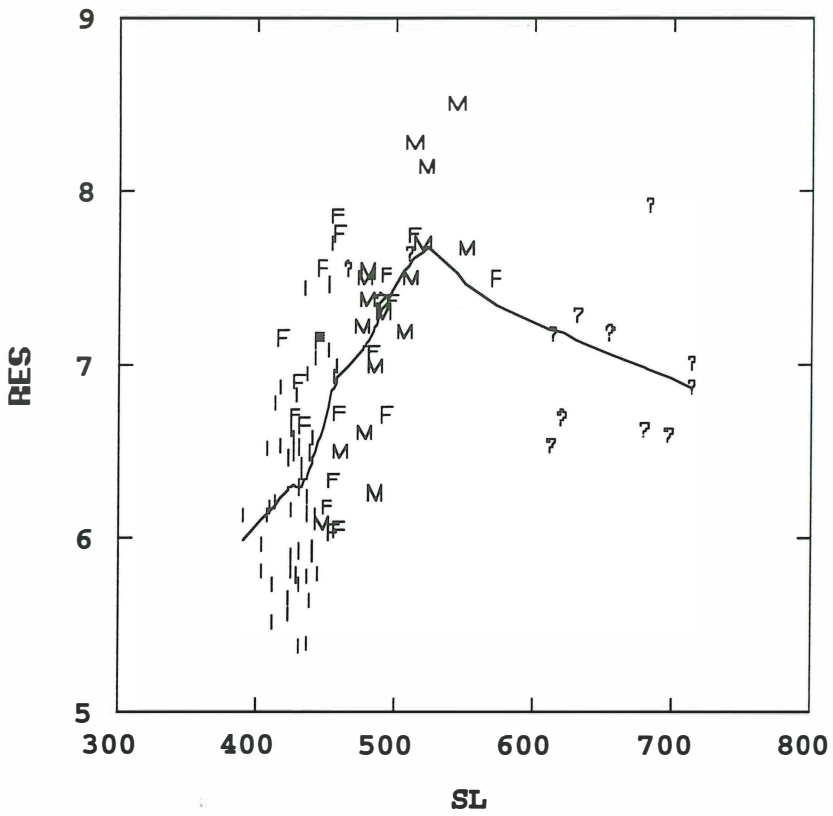

Fig 4.3 . Relative eye size (RES) plotted against standard length (SL, mm), with juveniles excluded. Lowess curve shown.

I=immature, F=female, M=male, ?=mature but unknown sex.

unavailable (gutted at sea) so only muscle-specific enzymes could be analysed.

A full allozyme genetic comparison of this second form of blue-eye against the normal blue-eye was carried out (Table 4.4). Sample sizes of the aberrant form were low: either two or five individuals per locus. However, in estimating genetic similarity or dissimilarity between morphs or species, the number of loci screened is more important in terms of reducing variance in the final estimates of similarity than the number of individuals screened per locus (Nei, 1978a; Gorman and Renzi, 1979). Thirty three enzyme loci were screened, and for fourteen of these the aberrant fish were homozygous or heterozygous for alleles not seen in blue-eye, and for a further three loci they were homozygous for alleles rare in blue-eye. The remaining seventeen loci showed allelic similarities to the normal blue-eye. Some gel photographs comparing the two forms for muscle LDH and muscle GPI are included in Bolch et al. (1993) (see the appendix). These genetic similarities and dissimilarities indicate the existence of two different but closely related blue-eye species.

Genetic distances and identities between the two species are given in Table 4.5. Genetic distance (D) takes a range of 0 (where two taxa are genetically identical) to infinity (where two taxa share no alleles), and genetic identity (I) an inverse range of 1 to 0. Genetic distance is estimated as $-\log_e I$ (Nei, 1972), and its variance may be approximately estimated as $(1-I)/(In)$ (where n is the number of loci examined, Nei, 1978b). The genetic distance between the two species is approximately 0.77, with a genetic identity of approximately 0.46. In comparison, the genetic distances and identities between the samples of blue-eye from different locations (Table 5.4) are 0.000 and 1.000 respectively, for both Nei's unbiased and standard estimators. This emphasises the lack of differentiation among the blue-eye samples, and the considerable degree of genetic differentiation of the new species from the blue-eye. The average heterozygosity per locus of the new species is about 6% which is similar to that of the blue-eye.

We have provisionally called the second species the "ocean blue-eye" (Bolch et al., 1993), and it has been provisionally identified as *Schedophilus labyrinthicus* McAllister and Randall. The ocean blue-

Table 4.4. Allele frequencies of blue eye and unrecognised trevalla species. n=numbers of fish examined.

Allele frequencies and sample sizes

Locus	Allele	blue eye	unknown species	Locus	Allele	blue eye	unknown species
*sAAT-1**	115	0.201	-	*FH**	100	1.000	1.000
	100	0.799	1.000		n	169	5
	n	681	5				
				*G3PDH-1**	100	1.000	-
*sAAT-2**	140	0.007	0.250		75	-	1.000
	100	0.986	0.500		n	193	2
	60	0.007	0.250				
	n	218	2	*G3PDH-2**	100	1.000	1.000
					n	251	5
*mAAT**	90	-	-				
	-30	0.046	-	*GAPDH-1**	100	1.000	1.000
	-100	0.954	1.000		n	193	5
	n	681	5				
				*GAPDH-2**	-100	1.000	1.000
*ACO**	D	0.125	-		n	193	5
	E	0.875	-				
	F	-	0.500	*GPI-1**	140	-	1.000
	G	-	0.500		130	0.021	-
	n	4	2		100	0.976	-
					65	0.003	-
*ADA**	125	0.054	1.000		n	266	5
	105	0.007	-				
	100	0.862	-	*GPI-2**	100	1.000	1.000
	95	0.056	-		n	266	5
	80	0.020	-				
	55	0.002	-	*IDDH**	140	-	1.000
	n	658	2		100	1.000	-
					n	193	5
*ADH**	55	0.001	-				
	-55	0.427	1.000	*IDH-1**	105	-	1.000
	-100	0.572	-		100	1.000	-
	n	609	2		n	193	5
*AK**	110	-	0.100	*IDH-2**	240	-	1.000
	100	1.000	0.900		100	1.000	-
	n	193	5		n	193	5
*CK-1**	100	0.995	1.000	*LDH-1**	100	1.000	1.000
	95	0.005	-		n	193	2
	n	217	2				
				*LDH-2**	100	1.000	1.000
*CK-2**	120	-	1.000		n	217	5
	100	1.000	-				
	n	193	5	*LDH-3**	100	1.000	-
					-50	-	1.000
*CK-3**	-100	1.000	1.000		n	217	5
	n	193	5				
				*sMDH-1**	130	0.002	-
*ESTD**	100	0.992	1.000		105	-	1.000
	85	0.005	-		100	0.998	-
	75	0.003	-		n	217	5
	n	185	5				

Table 4.4, continued

Locus	Allele	blue eye	unknown species
*sMDH-2**	185	-	1.000
	100	0.998	-
	0	0.002	-
	n	217	5
*ME1**	100	1.000	1.000
	n	193	5
*MPI**	115	0.013	-
	100	0.969	-
	85	0.017	1.000
	n	260	2
*PEP2**	105	0.026	-
	100	0.859	-
	90	0.114	-
	85	0.001	-
	80	-	0.900
	75	-	0.100
	n	681	5
*PGDH**	135	-	0.300
	120	-	0.600
	115	0.004	-
	100	0.829	0.100
	65	0.167	-
	n	681	5
*PGM-2**	500	0.001	-
	350	0.029	-
	100	0.963	-
	0	-	1.000
	-150	0.007	-
	n	678	5
*SOD1**	115	-	1.000
	100	1.000	-
	n	146	2
*XDH**	115	-	1.000
	100	1.000	-
	n	169	2

eye resembles the blue-eye in general appearance, but differs in fin-ray counts and in dorsal-fin and head shape (Table 4.6). Photographs of the two species are included in Bolch et al., 1993, see appendix. The ocean blue-eye is very closely related to a species fished in the northern Atlantic Ocean and Mediterranean Sea, *S. ovalis* (Cuvier and Valenciennes). However, these two species do differ in some fin ray counts and their relationships need to be established.

The distribution of the ocean blue-eye in Australian waters is unclear. While it may be quite common off New South Wales, we examined nearly 600 blue-eye from more southerly waters (off Tasmania, the Cascade Plateau, and South Australia) during the course of the genetic stock structure study, and found no evidence of its existence there. Either it is rare or absent from waters south of New South Wales (anecdotal evidence suggests the former as some fishermen say they catch specimens occasionally off Tasmania). Likewise, its distribution further north is unknown.

Juveniles of *S. labyrinthicus* are planktonic in the open ocean and the species may be widely distributed in warm-temperate latitudes of the Southern Hemisphere (Ahlstrom et al., 1976). So far it is known from the Pacific Ocean between Australia and the Easter Islands, including Lord Howe Island and the Gascoyne Seamount off New South Wales (McAllister et al., 1975). A specimen speared by a diver off the North Island of New Zealand (McDowall, 1982) and specimens recently identified by Japanese biologists (Amaoka et al., 1990) from off New Zealand as Atlantic trevalla (*H. moselii*) are undoubtedly this species. The adult form of the ocean blue-eye is known from only a few individuals world-wide. Little else is known of its biology, although spawning fish have been caught at the Gascoyne Seamount off New South Wales in July (AFZ observers).

4.4 Discussion

Evidence from this study supports the presence of two species of blue-eye within the fishery. These overlap in their distribution off New South Wales, although the relative contribution of each to the northern component of the fishery needs to be determined.

Table 4.5. Genetic distances and similarities between the blue-eye and the ocean blue eye

Nei (1978b) unbiased genetic distance: 0.768, standard error = 0.187

Nei (1972) standard genetic distance: 0.776, standard error = 0.189

Nei (1978b) unbiased genetic identity: 0.464

Nei (1972) standard genetic identity: 0.460

Note: the unbiased estimators take account of sample size.

Table 4.6. Features distinguishing the two blue-eye species

	Ocean blue eye *(S. labyrinthicus)*	Common blue eye *(H. antarctica)*
Dorsal fin	7-9 spines 26-29 rays Spines grading evenly in length into rays (last spine only slightly shorter than first ray)	9 spines 18-21 rays Spines not grading into rays (last spine much shorter than first ray)
Anal fin	3 spines 18-19 rays	3 spines 13-16 rays
Upper Jaw	Short (barely reaching past front of eye)	Long (reaching back to mid-eye or beyond)
Head	Compressed, narrow head with very blunt snout and rounded forehead	Broad, bulky head; larger fish have a flattened forehead; snout blunt to slightly rounded.

Two forms of the common blue-eye, differing in morphology and colour, appear to be related to growth factors which may reflect life history changes. The conclusion from these analyses is that the "small eye" and "big eye" forms represent two life history stages with metamorphosis between the two taking place at about 400–500 mm SL. Changes in eye colour, from yellowish to bluish, and in body colour, from a paler to a darker colour, are all parts of this developmental process.

Relative eye size in blue-eye declines in juvenile fish, at least for the interval 28 to 90 mm. Eye size is usually relatively greater in juvenile fish, so this decline is to be expected. No specimens between 90 mm and 390 mm have ever been recorded, so changes in relative eye size in this interval remain unknown. However, between 390 and about 500 mm relative eye size increases sharply, a change in gradient that is very unusual in fish. The literature is laden with data showing that fishes' eyes become proportionally smaller as the fish gets bigger. This is exemplified in Figs. 4.4 and 4.5, which present data from a parallel study on the orange roughy, *Hoplostethus atlanticus* (Elliott, unpublished).

The observed changes in eye morphometry are likely to be associated with a change in preferred habitat as the fish matures. Small juveniles (less than 90 mm SL) are associated with floating mats of seaweed (Last et al., 1993, and unpublished), while adults are bathypelagic or demersal at depths of 200-900 m. Light intensities at depths greater than 200 m are lower than those in surface waters, so a relatively large eye in adults may be an adaptation to cope with reduced light levels. Many other fish experience less dramatic changes in habitat as they mature, and for these species a continuous decline in relative eye size is normal. For example in the case of orange roughy, both juveniles and adults occupy demersal habitats.

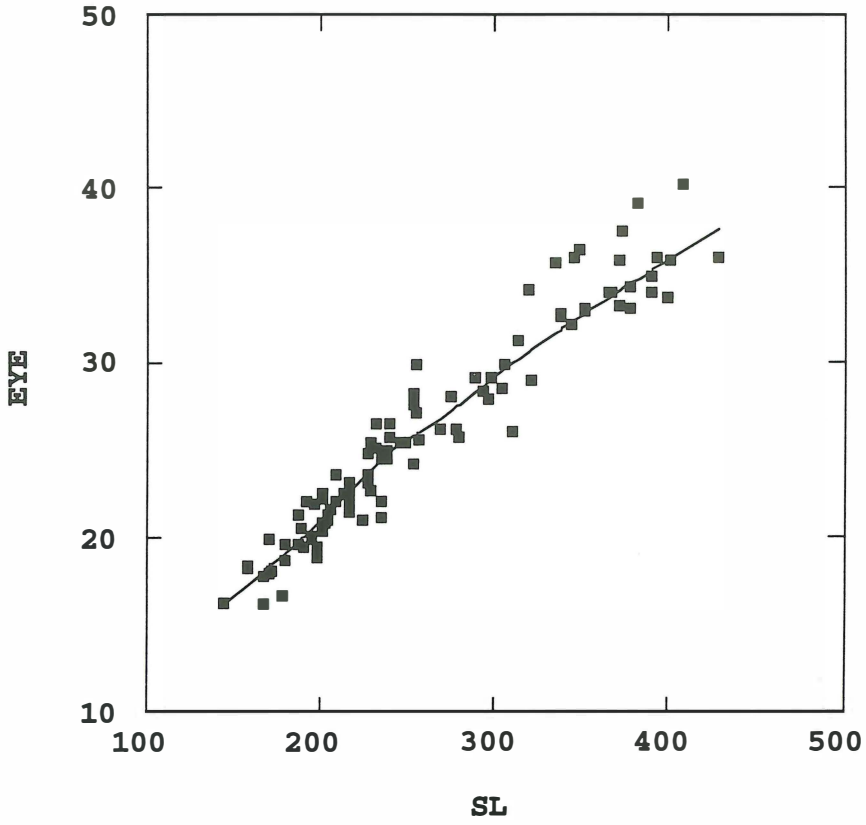

Fig. 4.4. Orange roughy: Eye diameter plotted against standard length (SL) , both in mm, with Lowess curve. (n=100)

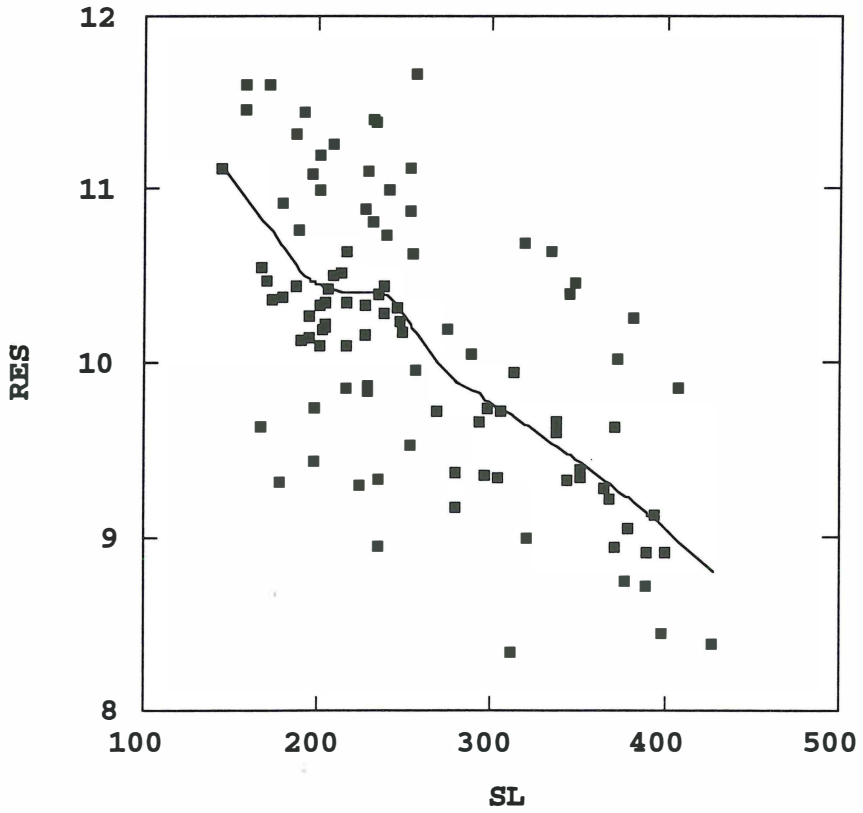

Fig 4.5. Orange roughy: Plot of relative eye size (RES) against standard length (SL), with Lowess curve. (n=100)

5. Stock structure of blue-eye

5.1 Introduction

There are many definitions of the word "stock", ranging from those that emphasize practical features such as production or management units, without necessarily any regard to biological integrity or significance, to those that emphasize genetic discreteness. Similarly, there are many approaches that have been used for determining stock extents and boundaries. Non-genetic methods include tagging studies, examinations of morphological and meristic variation, examinations of calcified structures such as scales or otoliths either by way of shape or chemical analysis, and determination of parasite loads. Genetic methods include studies of chromosome structure and examinations of DNA variability, either directly by nucleotide sequencing or restriction enzyme analysis, or indirectly by analysis of the protein products of genes. It is the latter method that was proposed and implemented for the present study. While other genetic methods such as mitochondrial DNA analysis should in principle provide a higher degree of resolution of population structure than allozyme analysis[1], such methods are more demanding and time-consuming than allozyme analysis, and in the relatively small blue-eye fishery may be judged too expensive. For the purposes of this work, we follow Ihssen et al.'s (1981) definition of stock as "an intra-specific group of randomly mating individuals with temporal or spatial integrity". Note that prior to this study, the only blue-eye stock structure work was Horn's (1989) work on tagged New Zealand blue-eye, referred to earlier (page 7).

Allozyme analysis involves the homogenisation of tissue in water or a buffered solution followed by centrifugation and application of the supernatant to a gel matrix. Note that different tissues have different enzymes active, and therefore screening several different tissues increases the number of enzymes and loci that can be examined. A number of samples, from different individuals, are placed

[1] Because mitochondrial DNA is haploid and maternally inherited, it has a population size only one quarter that of nuclear DNA assayed by allozymes: it is thus more sensitive to genetic drift effects than nuclear DNA and may reveal finer levels of population structure than that shown by allozyme analysis.

side by side and a current passed through the gel. Proteins, being strings of amino-acids, are electrically charged particles and move in response to a current. At pHs of around seven or eight, which are the normal buffer pHs used during electrophoresis, most proteins are negatively charged and migrate towards the anode. At the termination of the electrophoresis run, the current is turned off and a histochemical stain specific for the protein in question applied to the gel matrix. Only proteins specific to that stain will induce a coloured precipitate and can thus be identified and visualised. Most of the proteins examined are enzymes, and include within the histochemical stain the substrate of the enzyme in question. Uniform staining bands across individuals indicates genetic uniformity for that protein, whereas variation between individuals typically is indicative of genetic variation. Genotypes can thus be allocated to the different phenotypes, and allele or gene frequencies determined. Further general information on allozyme procedures can be found in Ferguson (1980), Whitmore (1990) and Hillis and Moritz (1990).

At the end of the experimental period, tables of gene frequencies in the different samples are inspected. Any significant differentiation among samples (once various correction procedures have been adopted which take into account the number of multiple tests performed) is indicative of a severe restriction of gene flow between such samples and indicates the existence of multiple stocks.

While the proposed work on stock delineation in blue-eye was to depend mainly on the genetic analysis described above, meristic data obtained from an investigation of "big eye" and "small eye" forms also permitted what could be regarded as a pilot study into the use of meristic characters for stock delineation in the blue-eye.

5.2. Materials and methods

Samples were taken from commercial dropline catches from six sites in southern Australian waters (Fig 5.1, Table 5.1). Several requests for samples from West Australia and New Zealand were unsuccessful. Samples of white muscle, liver and eye tissue were collected in one of three ways: (1) whole fish were stored on wet ice for 24 h after catching and tissue samples were dissected upon arrival

Figure 5.1 : Location of sites at which trevalla samples were collected.

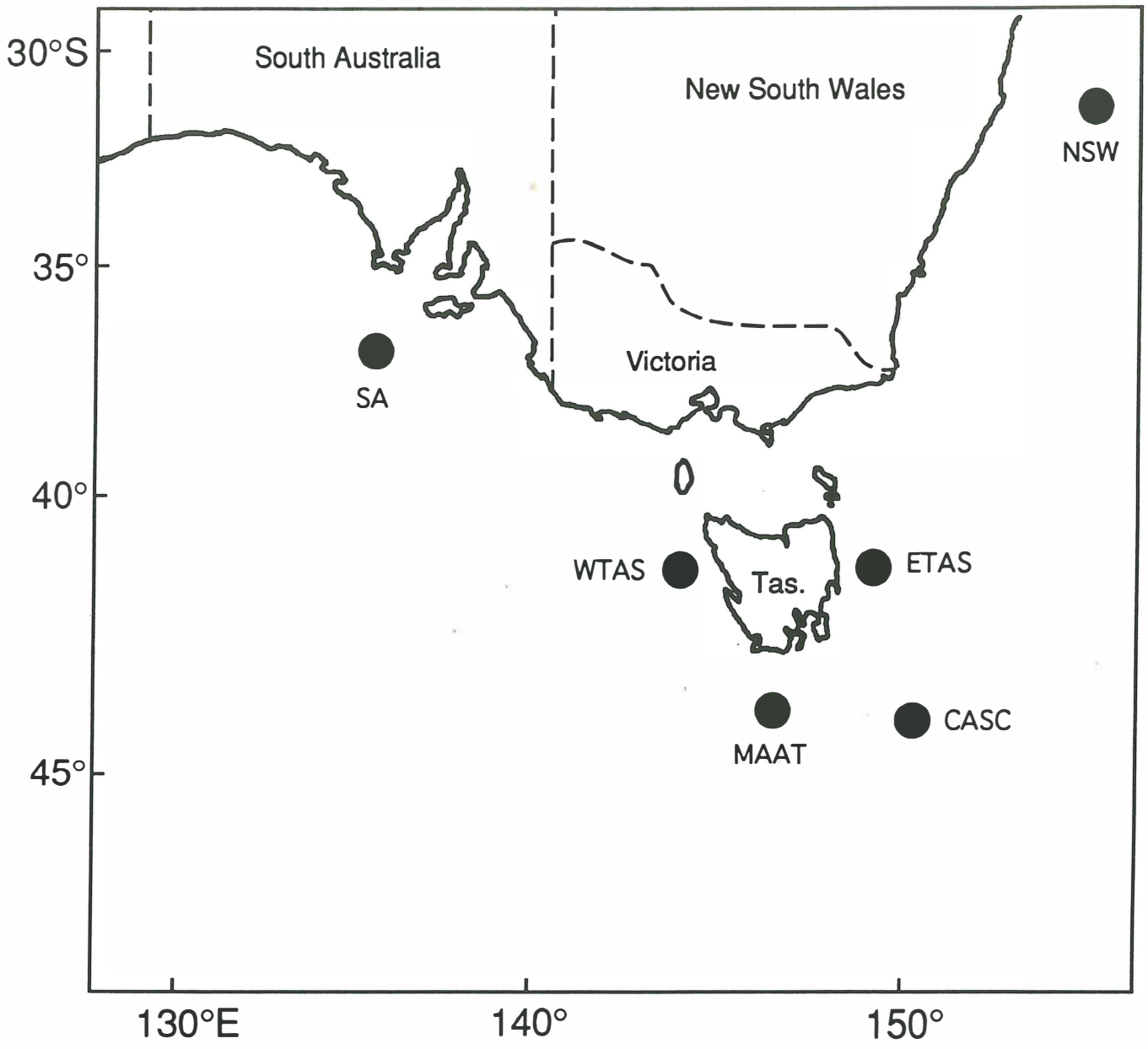

Table 5.1.Details of samples collected.

Area	Abbreviation	Approximate Locality	Date Collected
South Australia	SA	South of Port Lincoln	February, 1992
West Tasmania*	WTAS	41° S, 144° E 42° S, 145° E	December, 1991 March, 1992
South of Maatsuyker Island, Tasmania*	Maat.	43° S, 146° E	December, 1991 February, 1992
St Helens, east coast of Tasmania. *	ETAS	41° S, 148° E	October, 1991 November, 1991
Cascade Plateau	Casc.	43° S, 150° E	December, 1991
Barcoo and Taupo Seamounts, off east coast of NSW.	NSW	32° S – 33° S, 156° E	March, 1992

* Two collections from the same area.

in the laboratory, (2) heads and guts of fish were frozen soon after capture and transported frozen at -20°C to the laboratory where tissue samples were taken, or (3) tissue samples were taken on board and frozen at -20°C for transport to the laboratory. All tissue samples were sealed in individual polythene bags, numbered, and stored at -80°C in the laboratory prior to analysis. Liver and eye samples were not collected from fish caught at Cascade Plateau. New South Wales samples, collected from Barcoo and Taupo seamounts, were mixed before arriving at the laboratories and hence could not be analysed as separate samples. The whole fish were collected from east Tasmania (n=47) and west Tasmania (n=50). The sex of mature specimens of these whole fish was recorded in order to test for significant associations between sex and allele frequencies.

Small pieces of liver, muscle or eye were placed in 1.5 ml microcentrifuge tubes, homogenised manually with a few drops of distilled water, and spun in a microcentrifuge (approx 11 000 g) for 2 minutes. Supernatants were used for electrophoresis.

Electrophoretic systems for each enzyme are identified in Table 5.2. Gel system A refers to Helena Titan III cellulose acetate plates run using a tris-glycine buffer system (0.02M tris, 0.192M glycine; see Hebert and Beaton 1989 for further details). Gel system B used 12% Sigma starch gels and a discontinuous histidine/citrate buffer system (gel buffer: 0.005M histidine HCl, adjusted to pH 7.0 with 0.1M sodium hydroxide; electrode buffer: 0.41M trisodium citrate, adjusted to pH 7.0 with 0.5M citric acid).

An initial survey was carried out on 47 fish collected from the St Helens area, eastern Tasmania (ETAS). The products of 36 loci were found to be clearly resolved (Table 5.2). Five enzymes, AH (aconitase), FBALD (aldolase), EST (esterase, using a-naphthyl acetate as substrate), GDA (guanosine deaminase) and a fast-migrating PGM (phosphoglucomutase) showed poor activity or gave inconsistent patterns and were not examined further. Where two or more loci coded for the same enzyme, loci were numbered from 1 for the enzyme migrating closest to the anode. Alleles were numbered according to the relative mobilities of their products to that of the most common allele,

Table 5.2. Details of loci screened and electrophoretic conditions

Enzyme	Locus	EC number	Enzyme structure*	Routine tissue	Gel system
Aspartate aminotransferase	*sAAT-1**	2.6.1.1	dimer	muscle	A
	*sAAT-2**		dimer	liver	A
	*mAAT**		dimer	muscle	A
Acid phosphatase	*ACP**	3.1.3.2	–	liver	B
Adenosine deaminase	*ADA**	3.5.4.4	monomer	muscle	B
Alchohol dehydrogenase	*ADH**	1.1.1.1	dimer	liver	A
Adenylate kinase	*AK**	2.7.4.3	–	muscle	B
Creatine kinase	*CK-1**	2.7.3.2	–	eye	B
	*CK-2**		–	muscle	B
	*CK-3**		dimer	muscle	B
Esterase (U.V, Umb. acetate)	*ESTD**	3.1.-.-	dimer	liver	B
Fumarate hydratase	*FH**	4.2.1.2		muscle	A
Glycerol-3-phosphate dehydrogenase	*G3PDH-1**	1.1.1.8	–	liver	B
	*G3PDH-2**		–	muscle	B
Glucose-6-phosphate dehydrogenase	*G6PDH**	1.1.1.49	–	liver	A
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH-1**	1.2.1.12	–	muscle	A
	*GAPDH-2**		–	muscle	A
Glucose phosphate isomerase	*GPI-1**	5.3.1.9	dimer	muscle	A
	*GPI-2**		–	muscle	A
Sorbitol dehydrogenase	*IDDH**	1.1.1.14	–	liver	A
Isocitrate dehydrogenase	*IDH-1**	1.1.1.42	–	liver	B
	*IDH-2**		–	muscle	B
Lactate dehydrogenase	*LDH-1**	1.1.1.27	–	eye	B
	*LDH-2**		–	muscle	B
	*LDH-3**		–	muscle	B
Malate dehydrogenase	*sMDH-1**	1.1.1.37	dimer	muscle	A
	*sMDH-2**		dimer	muscle	A
Malic enzyme	*ME1**	1.1.1.40	–	muscle	A
Mannose phosphate isomerase	*MPI**	5.3.1.8	monomer	liver	A
Octanol dehydrogenase (2-octanol substrate)	*ODH**	1.1.1.73	dimer	liver	A
Amino peptidase (Leu-gly-gly)	*PEP1**	3.4.-.-	dimer	muscle	A
(Leu-Tyr)	*PEP2**	3.4.-.-	dimer	muscle	A
6–Phosphogluconate dehydrogenase	*PGDH**	1.1.1.44	dimer	muscle	B
Phosphoglucomutase	*PGM-2**	5.4.2.2	monomer	muscle	A
Superoxide dismutase	*SOD1**	1.15.1.1	–	liver	A
Xanthine dehydrogenase	*XDH**	1.2.3.2	–	liver	A

* Inferred from heterozygote banding patterns

designated 100. Proteins that migrated towards the cathode rather than the anode were given negative allele designations (e.g. *ADH**-100). At least 24 individuals from each of the six areas were screened for the 36 loci, and all individuals from all areas (except Cascade Plateau, where liver and eye samples were not collected) were screened for the seven loci with average heterozygosities greater than 0.06 (*sAAT-1**, *mAAT**, *ADA**, *ADH**, *PEP-2**, *PGDH**, *PGM-2**).

Chi-square tests for conformance to Hardy-Weinberg equilibrium were carried out using BIOSYS-1 (Swofford and Selander, 1989). Rare alleles were pooled where appropriate to reduce the number of classes with expected values of five or less, and tests were only carried out when the minimum expected value for any genotype exceeded one. Levene's (1949) correction for small sample sizes was used. Where multiple tests were carried out, for example when testing seven loci for goodness-of-fit to Hardy-Weinberg expectations, the standard Bonferroni procedure was applied (Miller, 1980; Lessios, 1992). The predetermined significance level, α (where $\alpha = 0.05$), was divided by the number of tests to obtain a corrected significance level, α'. A result was considered significant at the 0.05 level if the test result had a probability less than α'. The more powerful sequential Bonferroni method of Hochberg (1988) was also employed, but in all cases gave the same conclusions.

As no liver or eye samples were collected from the Cascade Plateau area, population genetic analyses were carried out either with all collection sites and 25 loci (liver and eye loci excluded) or with Cascade Plateau excluded but using all 36 loci.

Allele frequency differentiation across samples was tested using 1000 randomisations of the Monte Carlo chi-square procedure of Roff and Bentzen (1989). Standard chi-square analyses are very sensitive to small expected values, and thus require that rare alleles be pooled. This pooling, with the consequent loss of information, is avoided in the Roff and Bentzen approach. The probability of obtaining a value of χ^2_{null} as large or larger than that obtained from the actual observations, χ^2, was given by $P=n/1000$, where n was the number of randomisations that generate $\chi^2_{null} \geq \chi^2$.

Differentiation among samples was quantified using Nei's gene diversity statistic G_{ST} (Nei, 1973), which reflects the proportion of the total genetic variation attributable to differentiation between populations or samples. Values of G_{ST} can range from 1, where samples share no alleles, to 0, where samples share the same alleles at the same frequency. For example, a G_{ST} value of 0.10 means that for that locus, 10% of the variation in allele frequencies is attributable to variation between samples, and 90% to variation within samples. For each locus, G_{ST} was estimated as $(H_T - H_S)/ H_T$, where H_T represents total heterozygosity and H_S is average (Hardy-Weinberg expected) population heterozygosity. Total G_{ST} was estimated from the average H_T and average H_S across all loci. Sampling error alone will generate a positive value for G_{ST}, as it is very unlikely that two samples drawn from the same population will have identical allele frequencies. The proportion or magnitude of G_{ST} generated by sampling error, which we have termed $G_{ST.null}$, was estimated using a bootstrapping program written by Ward (unpublished), given the observed allele frequencies and sample sizes. This program has been earlier used in an analysis of orange roughy genetic data (Elliott and Ward, 1992). Simulations were run 1000 times to provide a mean value of $G_{ST.null}$ and a standard deviation. The probability of obtaining a value of $G_{ST.null}$ as large or larger than that obtained from the actual observations, G_{ST}, was given by $P=n/1000$, where n is the number of randomisations that generate $G_{ST.null} \geq G_{ST}$. Values of P less than 0.05 indicated significant differentiation between areas that could not be explained by sampling error alone.

An estimate of the number of migrants per generation (N_em) was derived from the relationship for the island model of migration (Wright, 1943):

$$F_{ST} = 1/(1 + 4N_em) \quad \text{or} \quad N_em = 0.25\ (1/ F_{ST} - 1),$$

where $F_{ST} = G_{ST}$. This relationship between F_{ST} or G_{ST} and N_em is approximately true if $m \ll 1$, $\mu \ll$ m, and if the populations are at equilibrium (N_e is the effective population size, μ is the mutation rate and m is the rate of gene flow per generation). It assumes selective neutrality of the different genotypes, permitting population differentiation to be attributed solely to genetic drift and migration.

5.3. Genetic evidence

In the 97 whole fish examined, 37 had mature gonads and could be unequivocally sexed. These fish comprised 17 males and 20 females. No loci showed a significant difference in allele frequencies between sexes (Table 5.3), and therefore sexes were pooled for all subsequent analyses.

Samples of frozen heads and guts received from the New South Wales area were found to contain three samples which differed morphologically from the remaining heads. These heads possessed a blunt snout, a shorter jaw, a relatively larger eye and a narrower head than typical the blue-eye form. Routine allozyme analysis identified fixed differences at several of the loci examined, indicating that the three heads were of a different species. This topic was discussed earlier in section 4.3.

Allele frequencies and sample sizes for the six areas sampled are shown in Table 5.4. Heterozygotes at all loci showed banding patterns consistent with the known subunit structure (eg. Ward et al., 1992). Multiple chi-square tests were carried out to determine whether allele frequencies differed between repeated collections at the same site. Only *PGDH** differed significantly (P=0.034) between the two collections at the ETAS site. After applying the Bonferroni adjustment, this difference was not significant.

Chi square tests of goodness of fit to Hardy-Weinberg expectations were carried out. The criterion applied for a test to be valid was that the minimum expected cell size should be greater than one. All loci were tested as di-allelic polymorphisms, pooling rare alleles when necessary. A total of 27 valid tests were carried out (Table 5.5). Only two showed significant deviation from expected genotype proportions, and these were only barely significant. They were *PEP-2** at Maatsuyker (P=0.047, n=79) and *PGDH** at Cascade Plateau (P=0.048, n=67). After applying the Bonferroni procedure for multiple tests, neither test was significant at the adjusted level of $\alpha' = 0.0019$ (where $\alpha' = 0.05/27$).

Table 5.3. Tests of associations between sex and allele frequencies of polymorphic loci

locus	allele	male (n=17)	female (n=20)	P
*mAAT**	-100	0.970	0.925	0.620
	-30	0.030	0.075	
*sAAT-1**	100	0.765	0.825	0.572
	115	0.235	0.175	
*ADA**	80	0	0.025	0.100
	95	0.030	0.050	
	100	0.970	0.800	
	125	0	0.125	
*ADH**	-55	0.353	0.475	0.348
	-100	0.647	0.525	
*PEP-2**	90	0.089	0.050	0.852
	100	0.882	0.925	
	105	0.029	0.025	
*PGDH**	65	0.176	0.175	1.000
	100	0.824	0.825	
*PGM-2**	100	0.941	0.950	1.000
	350	0.059	0.050	

Probabilities (P) estimated using Fisher's exact 2x2 test for *mAAT**, *sAAT-1**, *ADH**, *PGDH**, and *PGM-2**, and Roff and Bentzen (1989) test using 1000 randomisations for *ADA** and *PEP-2**

Table 5.4. Allele frequencies and numbers of fish (n) examined.

		Allele frequencies and sample sizes					
Locus	Allele	SA	WTAS	Maat.	ETAS	Casc.[1]	NSW
*sAAT-1**	115	0.222	0.220	0.196	0.185	0.179	0.188
	100	0.778	0.780	0.804	0.815	0.821	0.813
	(n)	(142)	(143)	(79)	(154)	(67)	(96)
*sAAT-2**	140	-	0.010	-	0.005	-	0.021
	100	1.000	0.990	1.000	0.979	-	0.979
	60	-	-	-	0.016	-	-
	(n)	(24)	(50)	(24)	(96)	(0)	(24)
*mAAT**	90	-	0.003	-	-	-	-
	-30	0.046	0.028	0.044	0.039	0.030	0.031
	-100	0.954	0.969	0.956	0.961	0.970	0.969
	(n)	(142)	(143)	(79)	(154)	(67)	(96)
*ACP**	100	1.000	1.000	1.000	1.000	-	1.000
	(n)	(47)	(50)	(24)	(24)	(0)	(24)
*ADA**	125	0.046	0.084	0.044	0.045	0.045	0.042
	105	0.011	0.007	0.006	0.003	-	0.005
	100	0.852	0.839	0.880	0.870	0.903	0.885
	95	0.063	0.045	0.051	0.062	0.030	0.057
	80	0.028	0.017	0.019	0.019	0.022	0.010
	55	-	0.007	-	-	-	-
	(n)	(142)	(143)	(79)	(154)	(67)	(96)
*ADH**	55	-	-	-	-	-	0.005
	-55	0.380	0.427	0.462	0.445	-	0.438
	-100	0.620	0.573	0.538	0.555	-	0.557
	(n)	(137)	(143)	(79)	(154)	(0)	(96)
*AK**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(47)	(50)	(24)	(24)	(24)	(24)
*CK-1**	100	1.000	1.000	1.000	0.986	-	1.000
	95	-	-	-	0.014	-	-
	(n)	(24)	(50)	(24)	(71)	(0)	(24)
*CK-2**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*CK-3**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*ESTD**	100	1.000	1.000	0.976	1.000	1.000	0.979
	85	-	-	0.024	-	-	-
	75	-	-	-	-	-	0.021
	(n)	(24)	(50)	(42)	(71)	(24)	(24)
*FH**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)

Table 5.4 continued

Locus	Allele	SA	WTAS	Maat.	ETAS	Casc.[1]	NSW
*G3PDH-1**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*G3PDH-2**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(105)	(24)	(24)
*G6PDH**	100	1.000	1.000	1.000	1.000	-	1.000
	(n)	(24)	(50)	(24)	(47)	(0)	(24)
*GAPDH-1**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*GAPDH-2**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*GPI-1**	130	-	-	-	0.025	-	-
	100	1.000	1.000	1.000	0.958	1.000	1.000
	65	-	-	-	0.004	-	-
	(n)	(24)	(50)	(24)	(120)	(24)	(24)
*GPI-2**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(120)	(24)	(24)
*IDDH**	100	1.000	1.000	1.000	1.000	-	1.000
	(n)	(24)	(50)	(24)	(47)	(0)	(24)
*IDH1-1**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*IDH1-2**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*LDH-1**	100	1.000	1.000	1.000	1.000	-	1.000
	(n)	(24)	(50)	(24)	(47)	(0)	(24)
*LDH-2**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(71)	(24)	(24)
*LDH-3**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(71)	(24)	(24)
*sMDH-1**	130	-	-	0.021	-	-	-
	100	1.000	1.000	0.979	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(71)	(24)	(24)
*sMDH-2**	100	1.000	1.000	0.979	1.000	1.000	1.000
	0	-	-	0.021	-	-	-
	(n)	(24)	(50)	(24)	(71)	(24)	(24)
*ME1**	100	1.000	1.000	1.000	1.000	1.000	1.000
	(n)	(24)	(50)	(24)	(47)	(24)	(24)
*MPI**	115	0.045	-	-	0.005	-	-
	100	0.933	0.990	1.000	0.979	-	0.958
	85	0.022	0.010	-	0.016	-	0.042
	(n)	(67)	(50)	(24)	(95)	(0)	(24)

Table 5.4. continued

Locus	Allele	SA	WTAS	Maat.	ETAS	Casc.[1]	NSW
*ODH**	120	0.015	0.010	-	-	-	0.021
	100	0.955	0.990	1.000	1.000	-	0.958
	70	0.030	-	-	-	-	0.021
	(n)	(67)	(50)	(24)	(47)	(0)	(24)
*PEP1**	100	1.000	1.000	1.000	0.989	1.000	1.000
	85	-	-	-	0.011	-	-
	(n)	(24)	(50)	(24)	(95)	(24)	(24)
*PEP2**	105	0.021	0.028	0.032	0.023	0.037	0.021
	100	0.838	0.888	0.848	0.847	0.881	0.859
	90	0.137	0.084	0.120	0.127	0.082	0.120
	85	0.004	-	-	0.003	-	-
	(n)	(142)	(143)	(79)	(154)	(67)	(96)
*PGDH**	115	0.004	-	-	0.010	-	0.005
	100	0.856	0.829	0.810	0.812	0.866	0.807
	65	0.141	0.168	0.190	0.179	0.134	0.188
	(n)	(142)	(143)	(79)	(154)	(67)	(96)
*PGM-2**	500	0.004	-	-	-	-	-
	350	0.039	0.014	0.038	0.029	0.052	0.016
	100	0.947	0.983	0.962	0.958	0.918	0.984
	-150	0.011	0.003	-	0.013	0.030	-
	(n)	(142)	(143)	(79)	(154)	(67)	(96)
*SOD1**	100	1.000	1.000	1.000	1.000	-	1.000
	(n)	(24)	(50)	(24)	(24)	(0)	(24)
*XDH**	100	1.000	1.000	1.000	1.000	-	1.000
	(n)	(24)	(50)	(24)	(47)	(0)	(24)

[1] No livers or eyes available for Cascade Plateau samples.

Table 5.5. Hardy-Weinberg tests of goodness-of-fit of observed genotype numbers to expected numbers. Values given are probabilities that samples are in Hardy-Weinberg proportions. Significant results ($P<0.05$) in bold.

locus	SA	WTAS	Maat.	ETAS	Casc.	NSW
*sAAT-1**	0.057	0.124	0.158	0.520	0.366	0.636
*ADA**	0.977	0.818	0.923	0.294	-[1]	0.831
*ADH**	0.616	0.706	0.906	0.910	-[2]	0.339
*PEP-2**	0.303	0.529	**0.047**	0.764	-[1]	0.890
*PGDH**	0.944	0.505	0.866	0.744	**0.048**	0.326

[1]Valid test not possible

[2]Locus not scored as no liver tissue available.

The genetic diversity of blue-eye, as measured by total heterozygosity (H_T) at the seven polymorphic loci, ranged from 0.491 for *ADH** to 0.071 for *AAT-1**. For the nine other variable loci, H_T varied from 0.055 for *MPI** to 0.004 for *PEP-1** (Table 4.6). Twenty loci were monomorphic. The mean H_T across all 36 loci was equal to 0.053.

Results of a series of heterogeneity chi-square tests on allelic frequency variation, utilising the Roff and Bentzen (1989) procedure, are shown in Table 5.6. Each of the 16 variable loci was analysed separately for all samples and the Bonferroni procedure for multiple tests applied using $\alpha = 0.05$ and $\alpha' = 0.0031$. This analysis did not reveal significant heterogeneity for any locus. Similarly, comparison of the two most geographically separated areas, South Australia and New South Wales, failed to show significant heterogeneity ($P>0.175$ for all loci, $P=0.601$ across all loci).

This minimal stock differentiation between samples was confirmed by the gene diversity analysis (Nei, 1973). G_{ST} values were never greater than 0.022 and were less than 0.004 for each of the seven most polymorphic loci (Table 5.6). Considering the bootstrap analysis, G_{ST} was greater than $G_{ST.null}$ for 8 of the 16 variable loci, but was significantly greater for only one locus (*PGM-2**, $P=0.023$). After applying the Bonferroni adjustment for multiple tests, and thereby setting $\alpha'= 0.0031$, no locus showed statistically significant differentiation (Table 5.6). The mean G_{ST} across all 36 loci was equal to 0.0038. This implies that only 0.38% of all the genetic variation observed arises from among sample differences, with the remainder or 99.62% of all variation existing within samples. However, this observed G_{ST} value is approximately the same as the mean 36 locus value of $G_{ST.null}$ of 0.0044 (SD = 0.0011). Thus this estimate of G_{ST} could have arisen solely from sampling error.

5.4 Morphological evidence

A total of 97 fish were analysed for meristic variation (Table 5.7). Forty seven of these came from the east coast of Tasmania (off St. Helens, by M. Tucker in October 1991) and 50 from the west coast (off Sandy Cape, by K. Krause in December 1991).

Table 5.6. Analysis of genetic diversity in south east Australian samples of blue eye trevalla.

		Heterogeneity chi-square analysis[1]		Gene diversity analysis[2]			$G_{ST\ .null}$ [3]		
Locus	No. of Pops.	χ^2	P	H_T	H_S	G_{ST}	mean	$S.D.$	P [4]
*sAAT-1**	6	5.666	0.901	0.0712	0.0711	0.0013	0.0040	0.0026	0.916
*sAAT-2**	5	6.083	0.648	0.0205	0.0203	0.0091	0.0121	0.0062	0.638
*mAAT**	6	2.149	0.829	0.3226	0.3221	0.0016	0.0036	0.0027	0.760
*ADA**	6	21.808	0.665	0.2347	0.2338	0.0036	0.0040	0.0018	0.532
*ADH**	5	9.189	0.264	0.4914	0.4898	0.0032	0.0035	0.0029	0.440
*CK-1**	5	3.455	0.786	0.0056	0.0056	0.0113	0.0098	0.0066	0.372
*ESTD**	6	18.032	0.048	0.0148	0.0145	0.0169	0.0112	0.0055	0.134
*GPI-1**	6	8.630	0.510	0.0097	0.0095	0.0214	0.0125	0.0062	0.073
*sMDH-1**	6	8.060	0.439	0.0069	0.0068	0.0174	0.0078	0.0070	0.180
*sMDH-2**	6	8.060	0.439	0.0069	0.0068	0.0174	0.0077	0.0075	0.191
*MPI**	5	16.657	0.027	0.0547	0.0537	0.0197	0.0105	0.0061	0.076
*ODH**	5	13.683	0.100	0.0380	0.0374	0.0165	0.0097	0.0052	0.084
*PEP1**	6	3.086	0.831	0.0035	0.0035	0.0088	0.0108	0.0070	0.620
*PEP2**	6	10.044	0.825	0.2467	0.2459	0.0033	0.0040	0.0023	0.552
*PGDH**	6	7.409	0.665	0.2836	0.2826	0.0036	0.0041	0.0027	0.487
*PGM-2**	6	22.976	0.066	0.0800	0.0792	0.0100	0.0040	0.0023	0.023
Across 25 loci				0.0532	0.0530	0.0038	0.0044	0.0011	0.684
Across 36 loci				0.0531	0.0529	0.0038	0.0044	0.0011	0.653

[1] Using the randomisation procedure of Roff and Bentzen (1989). See text.

[2] Nei (1973).

[3] See text

[4] Probability estimated from number of cases in 1000 bootstrap replicates where $G_{ST\ .null} \geq G_{ST}$

Table 5.7. Morphometrics and meristics of blue eye trevalla from the east and west coasts of Tasmania. Dorsal fin spines and anal fin spines were invariant, at 9 and 3 respectively.

Trait		east coast	west coast	*P*
standard length (mm)	mean	457.660	441.680	0.026
	n	47	50	
	SD	43.493	23.539	
dorsal fin rays	mean	19.553	19.673	0.423
	n	47	49	
	SD	0.686	0.774	
anal fin rays	mean	14.578	14.800	0.032
	n	45	50	
	SD	0.543	0.452	
left pectoral rays	mean	20.468	20.380	0.467
	n	47	50	
	SD	0.584	0.602	
right pectoral rays	mean	20.426	20.420	0.967
	n	47	50	
	SD	0.683	0.642	
left gill rakers	mean	24.936	25.320	0.042
	n	47	50	
	SD	1.009	0.819	
right gill rakers	mean	24.977	25.320	0.070
	n	43	50	
	SD	0.913	0.891	
external eye diameter (mm)	mean	32.004	28.775	0.003
	n	43	50	
	SD	6.579	3.394	
RES (relative eye size)	mean	6.877	6.504	0.012
	n	43	50	
	SD	0.835	0.558	

The average size of the fish from the east coast sample was slightly greater than that of the west coast sample, with an appreciably larger external eye diameter and relative eye size. However, these are small samples and not necessarily representative of the overall commercial catch. Since we had to pay market prices for fish (about $4 per kg), and since we needed fish primarily for genetic and meristic purposes, our samples from both coasts may have been biased somewhat towards the smaller fish. However, this did not bias the meristic data because meristic characters are not altered by size.

Of the six characters compared between the east and west coast samples, two (anal-fin rays and left gill rakers) were just significant at the 5% level, with a third (right gill rakers) verging on being significant. However, these six characters are not all independent of each other. Of the 15 possible pairwise comparisons, two are significantly correlated at the 5% level after allowing for Bonferroni adjustment of probability levels for multiple tests. These two comparisons are left pectoral rays versus right pectoral rays ($P<0.001$), and left gill rakers versus right gill rakers ($P<0.001$). Dorsal and anal fin rays were nearly significantly correlated at the 5% level ($P=0.088$). In order to circumvent this problem of non-independence of left and right characters, tests were carried out comparing total pectoral ray counts (which equal the sum of the left and right counts) and total gill raker counts between the two samples. The results were:

Trait		east coast	west coast	*P*
total pectoral rays	mean	40.894	40.800	0.695
	n	47	50	
	SD	1.202	1.143	
total gill rakers	mean	49.907	50.640	0.035
	n	43	50	
	SD	1.823	1.480	

Thus, two (anal fin rays, total gill rakers) of the four independent meristic traits showed evidence of differences between east and west coast fish. However, both these traits were only just significant at

the 0.05 level (P=0.032, P=0.035), and following the conservative Bonferroni approach of adjusting the 0.05 significance level for the number of tests, neither is significant at the adjusted 0.05 level of 0.0125 (calculated from 0.05/4).

An alternative means of assessing the overall significance of these tests is to combine the four probabilities (0.423, 0.032, 0.695, 0.035) from these independent tests as outlined by Sokal and Rohlf (1981, p 780). This yields a chi-square value of 16.0373 with 8 degrees of freedom, equivalent to a probability of 0.042, and indicates that there is a (just) significant difference between the meristic counts of blue-eye from the east and west coasts of Tasmania. At the moment, considering the small sample sizes and the marginal statistical significance of the results, we consider these data only suggestive of a possible difference between fish from the two Tasmanian coasts.

5.5 Discussion

The average heterozygosity per locus of blue-eye at about 5% per locus is typical of fish in general (Ward et al., 1992) and marine fish in particular (Smith and Fujio, 1982; Ward et al., 1993). Marine fish frequently show low levels of genetic subpopulation differentiation, but the value of G_{ST} recorded here for blue-eye (a maximum of 0.0038) is an order of magnitude less than the average of 57 species of marine teleosts previously studied (0.062, Ward et al., 1993). Our survey detected no evidence of genetically isolated subpopulations in the southern Australian region, although only a small portion of the geographic range of blue-eye was covered. Samples from seamounts off the New South Wales coast could not be distinguished from Tasmanian samples nor from a sample from the Great Australian Bight. A G_{ST} value of 0.0038 would equate to an N_em value of around 65 migrants per generation per locality, but given that this level of G_{ST} can be accounted for simply by sampling error, numbers of migrants are likely to be much higher than this. It would appear either that gene flow between areas is sufficient to maintain a very high degree of subpopulation homogeneity, or that divergence among blue-eye subpopulations is recent and too limited to be detected by the present study.

A similar lack of genetic stock structure following allozyme electrophoresis of trawled fish species in the southeastern region of Australia has been reported for the blue grenadier (Milton and Shaklee, 1987), orange roughy (Elliott and Ward, 1992) and jackass morwong (Richardson, 1982; Elliott and Ward, 1993b). However, mitochondrial DNA analysis discriminated New South Wales roughy from those in more southerly Australian waters (Smolenski et al., 1993) and both allozyme and mitochondrial DNA data showed significant differences between Australian and New Zealand samples of morwong (Elliott and Ward, 1993; Grewe et al., unpublished). It remains possible that subpopulations of blue-eye may be differentiated by further genetic studies based on more extensive sampling of fish or by mitochondrial DNA studies.

The locations of blue-eye spawning areas in Australian waters are unknown, although around Tasmania, mature fish are seen on the east coast between January and March (P. Baelde, Div. of Sea Fisheries Tasmania, pers. comm.). The reproductive biology and development of the blue-eye is so poorly understood that the reasons underlying the genetic homogeneity of the blue-eye in southeastern Australia remain unclear; the homogeneity could result from larval, juvenile or adult migration, or from a combination of all these processes.

The recent discovery that floating mats of seaweed harbour juvenile blue-eye (page 6, also see Last et al. (1993) in the appendix) suggests that these young stages may be a significant vector for gene flow. The young fish may be associated with the mats for several months, and during this time the mats may be moved considerable distances by winds or currents.

The meristic data is suggestive, but only suggestive, of possible differentiation of fish from the east coast and west coast of Tasmania. Meristic features such as fin rays and vertebrae are established early on in embryonic development, and at that time are at least partially dependent on environmental conditions (Taning 1952; MacGregor and MacCrimmon 1977). In general, higher temperatures promote faster development and reduced meristic counts, and therefore meristic counts can vary from year class to year class, even among fish restricted to a single locality. In order to make full use of

meristic data, it is important that fish be allocated to year classes and that specific year classes be compared between localities. Any follow up study will therefore not only need to determine year class, but will need much larger sample sizes in order to survey adequately individual year classes.

Such considerations further complicate the interpretation of these preliminary meristic results. Assuming that there were indeed significant differences between the fish from the east and west coasts, such differences could reflect a slightly different mixture of age classes in the two samples, and need not be indicative of true geographic differentiation. Even if the differences did reflect geographic differentiation, the very large degree of overlap in the values of those two characters showing differences (gill rakers and anal-fin rays) still means that there could be a high degree of mixture of individuals originating from the two different areas. Really, all that can be said at this stage is that meristic analysis has some potential for stock structure analysis in blue-eye.

Prior to this study, the only information concerning stock structure in blue-eye was gained from short term tagging studies carried out in New Zealand waters (Horn, 1989). This showed that 36 out of 40 tagged and recaptured specimens were caught on the grounds on which they were tagged, although one fish moved 490 km in 137 days and a second fish moved 450 km in 231 days. Horn (1989) concluded that while most individuals are fairly sedentary in the short term (6-8 months), the species is capable of long and fairly rapid migrations. Thus gene flow among Australian subpopulations may be mediated both by active adult migrations and by passive mat-associated drift of juveniles.

Despite indications from this study that there is significant gene flow between Australian subpopulations, the slow growth rate of adult fish (> 3 yrs) raises the potential for depletion of local fishing areas. Australian stocks appear to be currently heavily exploited, as evidenced by the declining average size of fish (Webb, 1979; Williams, 1993); localised depletion has almost certainly occurred in some fishing areas (Williams, 1993). Given a reduction in fishing effort, the significant gene flow between areas indicated by the present study means that such areas should eventually recover by recruitment from other areas. Clearly, management of the blue-eye fishery should closely monitor current and future fishing effort.

A reliable assessment of biomass is urgently required to assess sustainable yield. Data gained from tagging studies and general biological information on age, growth and mortality would allow more rigorous interpretations to be drawn from the present genetic study.

6. General Discussion

This project had two major objectives:

* To investigate species composition of blue-eye in southern Australian waters through a detailed morphological and genetic comparisons of two identifiable morphs, and, if two species do exist, to prepare guideline information on their spatial and geographic distributions.

* To assess stock structure and to determine the approximate extent of gene flow among populations taken from different areas of southern Australia.

The two identifiable morphs which formed the focus of the first objective were shown very clearly to represent growth stages of a single species. The two morphs formed part of a continuum of morphological changes, which were almost certainly related to habitat change as fish developed from a surface living juvenile stage to the demersal adult form.

However, while these two forms were shown to be developmental stages of a single species of blue-eye, a distinct and previously unidentified second trevalla species was discovered from waters off New South Wales. This species is morphologically and genetically distinct from the common blue-eye. It has been called the "ocean blue-eye" and is provisionally identified as *Schedophilus labyrinthicus*. It seems that sometimes catches of this second species off New South Wales seamounts can be significant (Rowlings, NSW Fisheries, per. comm.), and there is a need to investigate the size and distribution of this resource. It may not make biological sense to include this species in a blue-eye TAC. The ocean blue-eye was not found amongst fish sampled from southern Australia, and based on its known distribution, it is likely to be rare at high latitudes.

Allozyme analysis of blue-eye failed to reveal genetic differences between samples taken from New South Wales, Tasmania and South Australia. Disappointingly, several attempts to collect samples from

West Australia and New Zealand failed. Seven polymorphic loci were screened in all fish sampled, totalling around 680 fish and averaging 114 per area, and 30 other less variable loci were screened in smaller samples of fish. In terms of sample sizes, this is a powerful genetics study, but as in all studies of its type, some important qualifications must be made. The only conclusion that can be drawn is that we have failed to reject the hypothesis that there is no genetic differentiation among areas. We are not entitled to claim that we have proven a hypothesis that "there is no genetic differentiation among areas". This hypothesis may well be true; there may be a single panmictic population in the southeast trawl region, but it is also possible that there were small but real differences between areas, which we failed to detect. With a statistical type II error set at 10% (i.e. allowing for the possibility of missing only 10% of significant cases of population differentiation), and with our sample sizes, only allele frequency differences greater than 0.2 would be regularly detected for average allele frequencies of around 0.7 (Richardson et al., 1986, page 62). In other words, even though our sample sizes are substantial, we would probably not be able to detect small differences in allele frequencies. Our sample sizes would have to be around four times larger to detect allele frequency differences of around 10%.

The fact that we failed to detect significant genetic differentiation suggest that there is significant gene flow among blue-eye populations in the region sampled, and it is therefore unlikely that fish targeted by dropline fishermen are genetically distinct from those targeted by mid-water trawling. It seems probable that mid-water trawling will therefore have impacts on the dropline fishery: the two fisheries cannot be considered as independent entities. However, the extent of gene flow among localities cannot be accurately quantified from the present genetic data. At least 65 individuals must migrate per population per generation in order to maintain levels of differentiation at the observed low values, but the actual numbers migrating could be much larger than this. Note also that the localities sampled were several hundred kilometres apart from each other and that adjacent localities will be expected to have far higher exchange rates.

Gene flow may be effected either by egg or juvenile drift, or by adult migrations. Both drift and active adult migration are likely to be important in the dispersal of blue-eye. New Zealand studies have shown

that while most individuals are relatively sedentary, adults are capable of moving several hundred kilometers in several months (Horn, 1989). Juveniles associated with floating mats of seaweed may likewise move considerable distances in the space of a few months, as the mat is blown by wind or carried by currents. Larvae spawned in one area could well mature into adults at some considerable distance from their natal site. For instance, Australian larvae may well recruit to New Zealand.

Gene flow between localities also carries with it the implication that localised depletion, whether caused by overfishing or natural fluctuations in abundance, is unlikely to be irreversible. Recoveries in stock abundance are likely, with recruitment coming either from surviving adults in that region or by immigration from outside. Such recoveries will clearly be assisted by curtailing fishing activities until stocks have reached such levels that sustainable fishing operations can recommence, a time interval that may be measurable in decades in severe cases of depletion. More precise estimates cannot be given because insufficient is known of the relative importance of self-recruitment and immigration, nor about fecundities and mortalities.

7. Implications and Recommendations

1. There is no evidence that blue-eye from separate areas form discrete reproductively isolated populations, and it is therefore unlikely that fish targeted by dropline fishing and mid-water trawling form distinct resources.

2. The lack of genetic differentiation among samples suggests but does not prove that blue-eye comprise a single panmictic population in south-east Australia. Present data are compatible with a range of scenarios ranging from extensive to limited but significant gene flow among localities. Given this uncertainty, a conservative approach to management seems appropriate.

3. The ocean blue-eye resource needs to be researched, and its extent and quantity determined. It should be separated in catch statistics from the common blue-eye and should not form part of the common blue-eye TAC.

The stock structure of blue-eye may be further clarified in a number of ways, all of which would necessitate substantial additional funds which might be deemed inappropriate in view of the relatively small size of the blue-eye fishery. In order of priority, we suggest these further investigations could include:

1). A mitochondrial DNA study could be carried out. Since this is a haploid molecule and is only transmitted by females, in principle mitochondrial DNA should show higher levels (approximately fourfold) of among sample differentiation than allozyme variation (which reflects nuclear DNA variability). Sample sizes would need to be around 100 fish per locality per polymorphic restriction enzyme.

2). Tagging studies could be implemented, perhaps using break-away hooks as used in the New Zealand study. Again, this is likely to be expensive and fraught with difficulties.

3). A large scale study of meristic variation could be implemented, which would need to be done in conjunction with an ageing analysis. A meristic study would need whole animals for accurate counts and purchase of fish on a sufficiently large scale could be prohibitively expensive. Re-sale of flesh after counting would decrease costs substantially, if it could be effected. A possible alternative would be to sample in conjunction with cooperative processors in various states.

4). Studies of parasites or otolith microchemistry could be undertaken. Both of these methodologies have shown promise in other species, though neither is yet regularly used in stock structure analyses.

5). The allozyme analysis could be extended. Only those loci described as polymorphic in this report need be investigated, although sample sizes of around 400 fish per locality would be needed to effect a substantial increase in rigor.

8. Acknowledgements

The principal investigators thank Chris Bolch (CSIRO) for his interest in and dedication to this project. We also thank Nick Elliott (CSIRO) for assisting with and contributing to this project in many ways. We also would like to thank the numerous blue-eye fishermen and processors who helped in the sampling process, namely Karl Krause, Mick Tucker, Charles Boulter, Eric Beir, Mike Rowley, Bill Hansen, Stingray Processors and Petuna Seafoods. The AFZ observer team, in particular Lisa Hick and Ian Peel, contributed in providing observations, kelp samples and juvenile specimens late in the project.

We also thank Howell Williams, Pascale Baelde (Tasmanian Division of Sea Fisheries), and Kevin Rowlings (NSW Fisheries) for their interest and for many useful discussions. Finally, we would like to thank FIRDC for funding this project.

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Appendices

1. Last, P., Bolch, C. and Baelde, P. 1993. Discovery of juvenile blue-eye. *Australian Fisheries* 52 (8), 16-17.

2. Bolch, C.J.S., Elliott, N.G. and Ward, R.D. 1993. Enzyme variation in south east Australian samples of the blue-eye or deepsea trevalla, *Hyperoglyphe antarctica* Carmichael 1818 (Teleostei: Stromateoidei). *Australian Journal of Marine and Freshwater Research*, in press.

3. Bolch, C., Last, P., Elliott, N., Ward, R.D. and Rowling, K. 1993. More trevalla meet the eye. *Australian Fisheries* 52 (4), 24-25.

4. Original application

Appendix 1

RESEARCH

Discovery of juvenile blue-eye

After more than a decade of searching, juvenile blue-eye trevalla were captured recently off Australia for the first time. Scientists from the CSIRO and Tasmanian Division of Sea Fisheries, Peter Last, Chris Bolch and Pascale Baelde report on this interesting discovery.

Members of the trevalla family (Centrolophidae), to which the blue-eye *(Hyperoglyphe antarctica)* belongs, have complex life histories and undergo dramatic shape changes during their growth.

In regions outside Australia, juvenile trevallas are known to live near the surface in association with floating objects, such as jellyfishes, salps, and other flotsam (Haedrich, 1967). Locally, juveniles of other trevalla species, such as the warehous *(Seriolella brama* and *S. punctata)*, have been observed living amongst the tentacles of the sea nettle *(Cyanea capillata)*. However, the location of juvenile blue-eye smaller than about half a metre total length has remained a mystery despite an expansion in exploratory fishing effort and larval sampling both off Australia and New Zealand since the 1970s.

Recent Fisheries Research and Development Corporation (FRDC) funded research has investigated the stock structure and biology of blue-eye off southern Australia (Bolch et al., 1993). The previous smallest known Australian specimens were about 410 mm fork length and weighed about 1.5 kg. Fish of this size aggregate near the surface where they can be caught by trolling and midwater trawling. Compared with the adult, these fish are generally paler, have a more evenly rounded forehead and a relatively smaller eye. This immature form appears to move to deeper water after reaching about 460 mm fork length (slightly less than 2 kg) where they are caught with adults by droplining over hard bottom on the upper continental slope.

Over recent years scientists and fishermen alike have speculated about the blue-eye's early life history. The apparent absence of very young fish in midwater has led to suggestions that they live in the adult habitat — close to rocky substrates in deep water. These habitats cannot be sampled effectively with fine-mesh or larval nets and their fauna is not well known.

In the northern hemisphere, juveniles of other *Hyperoglyphe* species are known to live near the surface under flotsam but are rarely found in association with jellyfish. The fast growing Atlantic blue-eye *(H. perciforma)* occurs in great abundance off the eastern USA during summer where it lives under drifting logs and other floating objects — this behaviour has led to it being called the 'barrelfish'. However, juveniles of other blue-eye species have proven more difficult to find. For example, the commercially important Japanese blue-eye *(H. japonica)* was marketed long before the juveniles were discovered (Abe, 1955).

The first Australasian specimens of juv-

Figure 2. Juvenile blue-eye amongst a drifting kelp mat. Photo: Thor Carter.

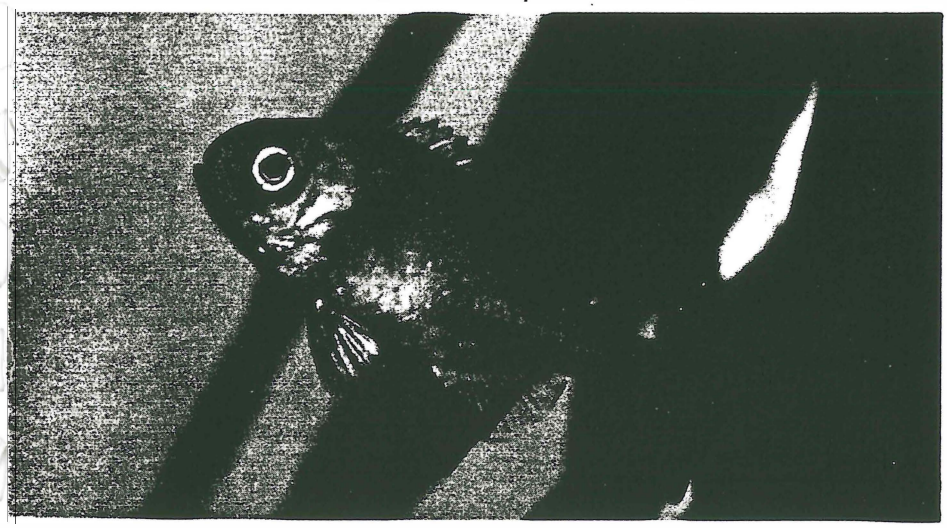


Figure 3. Adult blue-eye.

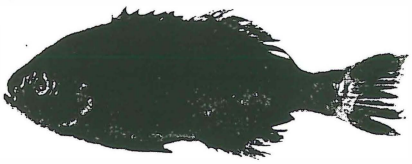
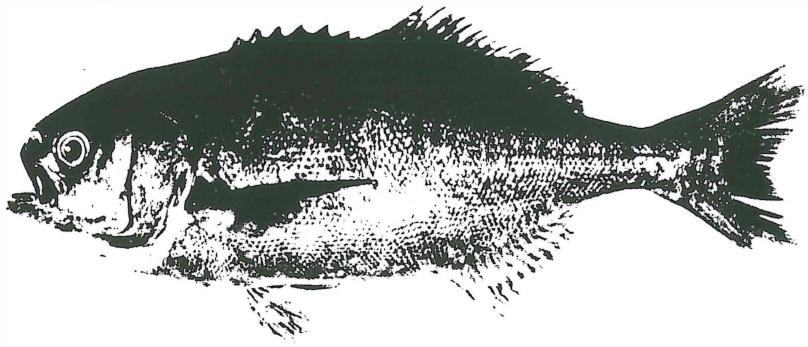


Figure 1. Juvenile blue-eye trevalla (Hyperoglyphe antarctica).



ile blue-eye trevalla were discovered cently during the processing of material llected by an AFMA Australian Fishing Zone Observer, Lisa Hick, beyond the elf break off eastern Tasmania. These h (Figure 1), which range in size from out 30–60 mm, were collected amongst a floating raft of the brown kelp, *Phyllospora comosa* (Figure 2), in mid-ne. Their discovery coincides with the e summer/autumn spawning off eastern Tasmania and their size indicates that the initial growth of the species is rapid.

Unlike some other trevallas, in which e juveniles barely resemble the adults in shape, young blue-eye are almost clones of the adult (Figure 3). Other trevalla juv-iles in the region including warehous, ay be more heavily spotted but are simi-lar to their adults in general colour. In comparison, blue-eye juveniles are uniform llowish brown in appearance, closely atching the colour of kelp in which they are found, whereas the adults are bluish or mauvish with a pale belly. The colour pat-rn of juvenile blue-eye is so well inte-ated with its habitat that its occurrence in kelp is likely to be real adaptation rather than a chance event.

Many juvenile trevallas use jellyfish for od as well as refuge (Haedrich, 1967). hey are able to occupy this potentially dangerous habitat through a combination f being partly resistant to the jellyfish's oxin, having a heavy mucous coating ver the skin, and through the simple avoidance of the tentacles. In Tasmanian waters, young warehou feed almost exclusively on jellyfish tentacles and are likely to gain protection from predators at the same time. Juvenile blue-eye, however, are likely to be concealed by camouflage from predators within the rafts but are unlikely to rely on the kelp as a direct food source. Instead, the stomachs of young blue-eye contained only copepods which may or may not occur in association with the kelp rafts.

The role of kelp in the life history of this trevalla may have an impact on the fishery. *Phyllospora* is one of the dominant kelps found in exposed nearshore habitats off Tasmania (Edgar, 1981). It belongs to a group of kelps , including the giant kelp *(Macrocystis)*, that have air bladders providing the fronds with buoyancy. These plants are often dislodged after heavy storms and form rafts which float on the surface and move at the mercy of winds and currents. If young blue-eye are dependent on these kelps for refuge, then the availability of rafts far from shore in the open sea may be a factor affecting their recruitment.

Several leading questions arise from this discovery. How common are juvenile blue-eye trevalla within these offshore kelp rafts? Are juveniles dependent on kelp rafts for their survival? If so which species of kelp? How common are these rafts beyond the shelf break? How long do the young fish remain within the rafts? Do juveniles feed only on copepods that co-occur within the rafts? Fishermen can play a valuable role in helping to answer these questions.

Young trevalla occur off our coasts during this time of year. Any additional specimens of juvenile blue-eye, or information on the type and frequency of kelp rafts beyond the shelf break, would be valuable in helping to answer these questions. Dip-nets are best for obtaining samples. All specimens should be frozen on capture and site information (that is, locality, collector and date) noted.

Please contact: Dr P. Baelde, Fisheries Research Laboratories, Crayfish Point, Taroona Tasmania 7053, tel (002) 27 7277 or Dr Peter Last, CSIRO Marine Laboratories, Castray Esplanade, Hobart 7000, Tasmania, tel (002) 32 5222. **The authors ackowledge the assistance of a FRDC grant.**

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Appendix 2

Aust. J. Mar. Freshwater Res., 1993, 44, 000–00

Enzyme Variation in South-eastern Australian Samples of the Blue-eye or Deepsea Trevalla, *Hyperoglyphe antarctica* Carmichael 1818 (Teleostei : Stromateoidei)

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Abstract

Six samples ($n = 67$ to 154) of blue-eye or deepsea trevalla were collected from south-eastern Australia (seamounts off New South Wales, a seamount south-east of Tasmania called the Cascade Plateau, off the east, south and west coasts of Tasmania, and off the coast of South Australia). All fish were analysed by starch or cellulose acetate electrophoresis for the products of seven polymorphic loci (defined in this study as those with an average heterozygosity greater than 0·06); a minimum of 24 fish per area were also analysed for 29 other less variable loci. The average heterozygosity per locus was 5·3%. Polymorphic loci showed no significant deviations from Hardy–Weinberg equilibrium. The coefficient of genetic subpopulation differentiation, G_{ST}, was 0·38%. Bootstrapping procedures showed that this low value could be attributed to sampling error alone. Contingency χ^2 analysis similarly failed to reveal any significant inter-sample differentiation for any locus. The results indicate that gene flow is sufficient to prevent any genetic differentiation among the sampled localities. During the course of the study a second trevalla species, *Schedophilus labyrinthicus*, was identified in the New South Wales component of the fishery.

Introduction

The blue-eye or deepsea trevalla (*Hyperoglyphe antarctica*) is thought to be widespread in the southern ocean, occurring in continental shelf and upper slope waters (100–600 m) off the coasts of southern Australia, New Zealand, South Africa, Tristan da Cunha in the southern Indian Ocean, and around many seamounts (McDowall 1982).

This species constitutes a small but significant hook fishery in south-eastern Australia, currently landing about 800 t annually with a market value in excess of $A3·6 million (H. Williams, Tasmanian Division of Sea Fisheries, personal communication). In recent years some demersal trawlers have diversified their operations to target trevalla aggregations in midwater and have taken appreciable quantities. Analyses of catch-per-unit-effort (CPUE) statistics in New Zealand have suggested a decline in abundance of trevalla in heavily trawled areas since the development of a trevalla/alfonsino midwater trawl fishery in 1983 (Horn and Massey 1989). As a consequence, there is concern among Australian hook fishers that midwater trawling in Australian waters will rapidly deplete stocks and thus threaten the viability of their traditional fishery. Management strategies now limit the amount of landed trawled catches of trevalla to 500 kg per trip.

Little is known about the biology of this species. Studies in southern Australian and New Zealand waters indicate that individuals grow very rapidly in the first few years of life, reaching fork lengths (LCF) $\leqslant 30$ cm in their first year and $\leqslant 60$ cm in their second year; there is a reduction in the rate of growth thereafter (Webb 1979; Jones 1985; Horn 1988).

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However, fish with LCF <45 cm are rarely caught by commercial vessels and, as with the juveniles of other stromateoid and *Hyperoglyphe* species (Haedrich 1967), the juvenile phase is most likely spent in surface waters (Horn and Massey 1989). Recruitment into both Australian and New Zealand commercial fisheries occurs at 2–3 years of age, with sexual maturity being reached at 6–7 years (Webb 1979). Most of the commercial catch consists of immature specimens (Webb 1979; Horn 1988).

Prior to the present study, there had been no attempts to assess the genetic stock structure of this species. Adult trevalla seem to be associated with rocky ground on the continental margin within a rather narrow depth band, and therefore trevalla stocks inhabiting seamounts may be separate from continental slope stocks. Additionally, there appear to be two distinct morphotypes of trevalla caught in Australian waters, distinguished by differing relative eye size, body colour and head shape, and commonly referred to as 'big-eyes' and 'small-eyes'. These 'morphs' of blue-eye trevalla may reflect intra- or inter-specific variation. Resolution of the taxonomic status of the two morphotypes and information on trevalla stock structure are crucial for effective management of a fishery under increasing exploitative pressures.

A variety of methods is available for studying stock structure, including tagging and otolith microchemistry studies, and meristic, morphometric, parasite and genetic analyses. Most genetic analyses of taxonomic or population structure issues examine either allozyme variation (which reflects nuclear DNA variation) or mitochondrial DNA variation. The aim of the present study was to examine allozyme variation in blue-eyue travalla in order to determine the two morphs represent one or two species, and whether there is genetic differentiation among samples collected from the continental shelf and seamounts in south-eastern Australian waters.

Materials and Methods

Samples were taken from commercial dropline catches from six sites in southern Australian waters (Fig. 1, Table 1). White muscle, liver and eye tissue were collected in one of three ways: (*1*) whole fish were stored on wet ice for 24 h after they were caught and tissue samples were dissected upon arrival in the laboratory; (*2*) heads and guts of fish were frozen soon after capture and transported at −20°C to the laboratory where tissue samples were taken; or (*3*) tissue samples were removed on board ship and frozen at −20°C for transport to the laboratory. All tissue samples were sealed in individual Polythene bags and stored at −80°C in the laboratory prior to analysis. Liver and eye samples were

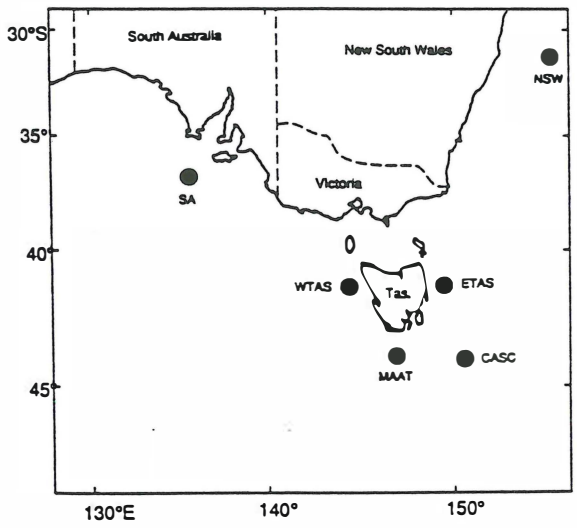

Fig. 1. Map of south-eastern Australia showing the areas from which samples were collected.

Table 1. Details of samples of *H. antarctica* collected in south-eastern Australia

Area	Abbreviation	Approximate locality	Date collected	No. of fish
South Australia	SA	37°S,133°E	February 1992	142
Western Tasmania[A]	WTAS	41°S,144°E	December 1991	50
		42°S,145°E	March 1992	93
South of Maatsuyker I., Tasmania[A]	MAAT	43°S,146°E	December 1991	28
			February 1992	51
St Helens, east coast of Tasmania[A]	ETAS	41°S,148°E	October 1991	47
			November 1991	107
Cascade Plateau	CASC	43°S,150°E	December 1991	67
Barcoo and Taupo Seamounts, east coast of NSW	NSW	32°S–33°S 156°S	March 1992	96

[A] Two collections from the same area.

not collected from fish caught at Cascade Plateau. New South Wales samples, collected from Barcoo and Taupo seamounts, were mixed before arrival at the laboratories and hence could not be analysed as separate samples. The whole fish were collected from eastern Tasmania ($n = 47$) and western Tasmania ($n = 50$). The sex of mature specimens of these whole fish was recorded in order to test for significant associations between sex and genotype frequencies.

Small pieces of liver, muscle or eye were placed in 1·5 mL microcentrifuge tubes, homogenized manually with a few drops of distilled water, and spun in a microcentrifuge (approximately 11 000 *g*) for 2 min. Supernatants were used for electrophoresis.

Electrophoretic systems for each enzyme are identified in Table 2. Gel system A refers to Helena Titan III cellulose acetate plates run using a Tris–glycine buffer system (0·02 M tris, 0·192 M glycine; see Hebert and Beaton 1989 for further details). Gel system B used 12% Sigma starch gels and a discontinuous histidine–citrate buffer system (gel buffer 0·005 M histidine HCl, adjusted to pH 7·0 with 0·1 M NaOH; electrode buffer 0·41 M trisodium citrate, adjusted to pH 7·0 with 0·5 M citric acid).

An initial survey was carried out on 47 fish collected from the St Helens area (ETAS). The products of 36 loci were clearly resolved (Table 2). Five enzymes, AH (aconitase), FBALD (aldolase), EST (esterase, using α-naphthyl acetate as substrate), GDA (guanosine deaminase) and a fast-migrating PGM (phosphoglucomutase) showed poor activity or gave inconsistent patterns and were not examined further. Where two or more loci coded for the same enzyme, loci were numbered from 1 for the enzyme migrating closest to the anode. Alleles were numbered according to the relative mobilities of their products to that of the most common allele, designated 100. At least 24 individuals from each of the six areas were screened for the 36 loci, and all individuals from each area (except Cascade Plateau, where liver and eye samples were not collected) were screened for the 7 loci with average heterozygosities greater than 0·06 (*sAAT-1**, *mAAT**, *ADA**, *ADH**, *PEP-2**, *PGDH**, *PGM-1**).

χ^2 tests for conformance to Hardy–Weinberg equilibrium were carried out using BIOSYS-1 (Swofford and Selander 1989). Rare alleles were pooled where appropriate to reduce the number of classes with expected values of 5 or less, and tests were only carried out when the minimum expected value for any genotype exceeded one. Levene's (1949) correction for small sample sizes was used. Where multiple tests were performed, the standard Bonferroni procedure was applied (Miller 1980; Lessios 1992). The predetermined significance level, α (where $\alpha = 0{\cdot}05$), was divided by the number of tests to obtain a corrected significance level, α'. A results was considered significant at the 0·05 level if the test result had a probability less than α'. The more powerful sequential Bonferroni method of Hochberg (1988) was also employed, but in all cases gave the same conclusions.

As no liver or eye samples were collected from the Cascade Plateau area, population genetic analyses were carried out either with all collection sites and 25 loci (liver and eye loci excluded) or with Cascade Plateau excluded but using all 36 loci.

Allele frequency differentiation across samples was tested using 1000 randomizations of the Monte Carlo procedure of Roff and Bentzen (1989). This procedures obviates the need to pool rare alleles.

Table 2. Details of *H. antarctica* loci screened and electrophoretic conditions

Enzyme	Locus	EC number	Enzyme structure[A]	Routine tissue	Gel system
Aspartate aminotransferase	*sAAT-1**	2.6.1.1	dimer	muscle	A
	*sAAT-2**		dimer	liver	A
	*mAAT**		dimer	muscle	A
Acid phosphatase	*ACP**	3.1.3.2	–	liver	B
Adenosine deaminase	*ADA**	3.5.4.4	monomer	muscle	B
Alcohol dehydrogenase	*ADH**	1.1.1.1	dimer	liver	A
Adenylate kinase	*AK**	2.7.4.3	–	muscle	B
Creatine kinase	*CK-1**	2.7.3.2	–	eye	B
	*CK-2**		–	muscle	B
	*CK-3**		dimer	muscle	B
Esterase (U.V, Umb. acetate)	*ESTD**	3.1.–.–	dimer	liver	B
Fumarate hydratase	*FH**	4.2.1.2		muscle	A
Glycerol-3-phosphate dehydrogenase	*G3PDH-1**	1.1.1.8	–	liver	B
	*G3PDH-2**		–	muscle	B
Glucose-6-phosphate dehydrogenase	*G6PDH**	1.1.1.49	–	liver	A
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH-1**	1.2.1.12	–	muscle	A
	*GAPDH-2**		–	muscle	A
Glucose phosphate isomerase	*GPI-1**	5.3.1.9	dimer	muscle	A
	*GPI-2**		–	muscle	A
Iditol dehydrogenase	*IDDH**	1.1.1.14	–	liver	A
Isocitrate dehydrogenase	*sIDHP**	1.1.1.42	–	liver	B
	*mIDHP**		–	muscle	B
Lactate dehydrogenase	*LDH-1**	1.1.1.27	–	eye	B
	*LDH-2**		–	muscle	B
	*LDH-3**		–	muscle	B
Malate dehydrogenase	*sMDH-1**	1.1.1.37	dimer	muscle	A
	*sMDH-2**		dimer	muscle	A
Malic enzyme	*MEI**	1.1.1.40	–	muscle	A
Mannose phosphate isomerase	*MPI**	5.3.1.8	monomer	liver	A
Octanol dehydrogenase (2-octanol substrate)	*ODH**	1.1.1.73	dimer	liver	A
Amino peptidase (Leu-gly-gly)	*PEP1**	3.4.–.–	dimer	muscle	A
(Leu-Tyr)	*PEP2**	3.4.–.–	dimer	muscle	A
6-Phosphogluconate dehydrogenase	*PGDH**	1.1.1.44	dimer	muscle	B
Phosphoglucomutase	*PGM-2**	5.4.2.2	monomer	muscle	A
Superoxide dismutase	*SOD1**	1.15.1.1	–	liver	A
Xanthine dehydrogenase	*XDH**	1.2.3.2	–	liver	A

[A] Inferred from heterozygote banding patterns.

The probability of obtaining a value of χ^2_{null} as large as or larger than that obtained from the actual observations, χ^2, was given by $P = n/1000$, where n was the number of randomizations that generate $\chi^2_{null} \geq \chi^2$ (see Table 3).

Differentiation among samples was quantified using Nei's gene diversity statistic G_{ST} (Nei 1973), which reflects the proportion of total genetic variation attributable to differentiation between populations. For each locus it was estimated as $(H_T - H_S)/H_T$, where H_T represents total heterozygosity and H_S is the average (Hardy–Weinberg expected) population heterozygosity. Total G_{ST} was estimated from the average H_T and average H_S across all loci. The proportion or magnitude of G_{ST} generated by sampling error, termed $G_{ST \cdot null}$ in the present study, was estimated using a bootstrapping program,

Table 3. Analysis of genetic diversity in south-eastern Australian samples of *H. antarctica*

Locus	No. of populations	Heterogeneity χ^2 χ^2	P^C	Gene diversity analysis[A] H_T	H_S	G_{ST}	$G_{ST.null}$[B] Mean	s.d.	P^D
*sAAT-1**	6	2·149	0·829	0·3226	0·3221	0·0016	0·0036	0·0027	0·760
*sAAT-2**	5	6·083	0·648	0·0205	0·0203	0·0091	0·0121	0·0062	0·638
*mAAT**	6	5·666	0·901	0·0712	0·0711	0·0013	0·0040	0·0026	0·916
*ADA**	6	21·808	0·665	0·2347	0·2338	0·0036	0·0040	0·0018	0·532
*ADH**	5	9·189	0·264	0·4914	0·4898	0·0032	0·0035	0·0029	0·440
*CK-1**	5	3·455	0·786	0·0056	0·0056	0·0113	0·0098	0·0066	0·372
*ESTD**	6	18·032	0·048	0·0148	0·0145	0·0169	0·0112	0·0055	0·134
*GPI-1**	6	8·630	0·510	0·0097	0·0095	0·0214	0·0125	0·0062	0·073
*sMDH-1**	6	8·060	0·439	0·0069	0·0068	0·0174	0·0078	0·0070	0·180
*sMDH-2**	6	8·060	0·439	0·0069	0·0068	0·0174	0·0077	0·0075	0·191
*MPI**	5	16·657	0·027	0·0547	0·0537	0·0197	0·0105	0·0061	0·076
*ODH**	5	13·683	0·100	0·0380	0·0374	0·0165	0·0097	0·0052	0·084
*PEP1**	6	3·086	0·831	0·0035	0·0035	0·0088	0·0108	0·0070	0·620
*PEP2**	6	10·044	0·825	0·2467	0·2459	0·0033	0·0040	0·0023	0·552
*PGDH**	6	7·409	0·665	0·2836	0·2826	0·0036	0·0041	0·0027	0·487
*PGM-2**	6	22·976	0·066	0·0800	0·0792	0·0100	0·0040	0·0023	0·023
Across 25 loci				0·0532	0·0530	0·0038	0·0044	0·0011	0·684
Across 36 loci				0·0531	0·0529	0·0038	0·0044	0·0011	0·653

[A] Nei (1973). [B] See text. [C] Using the randomization procedure of Roff and Bentzen (1989); see text. [D] Probability estimated from number of cases in 1000 bootstrap replicates where $G_{ST.null} \geq G_{ST}$.

given the observed allele frequencies and sample sizes (Elliott and Ward 1992). Simulations were run 1000 times to provide a mean value of $G_{ST\cdot null}$ and a standard deviation. The probability of obtaining a value of $G_{ST\cdot null}$ as large or larger than that obtained from the actual observations, G_{ST}, was given by $P=n/1000$, where n is the number of randomizations that generate $G_{ST\cdot null} \geq G_{ST}$. Values of $P<0{\cdot}05$ indicated significant differentiation between areas that could not be explained by sampling error alone.

An estimate of the number of migrants per generation ($N_e m$) was derived from the relationship for the island model of migration (Wright 1943):

$$F_{ST} = 1/(1+4N_e m) \quad \text{or} \quad N_e m = 0{\cdot}25\ (1/F_{ST} - 1)\,,$$

where $F_{ST} = G_{ST}$. This relationship between F_{ST} or G_{ST} and $N_e m$ is approximately true if $m \ll 1$, $\mu \ll m$, and if the populations are at equilibrium (N_e is the effective population size, μ is the mutation rate and m is the rate of gene flow per generation). It assumes selective neutrality of the different genotypes, permitting population differentiation to be attributed solely to genetic drift and migration.

Results

In the 97 whole fish examined, 37 had mature gonads and could be unequivocally sexed. These fish comprised 17 males and 20 females. None of the polymorphic loci showed a significant difference in allele frequencies between sexes, and therefore both sexes were pooled for all subsequent analyses.

These whole fish covered a wide range of relative eye sizes from the typical 'small-eye' morphotype to typical 'big-eye' morphotype. No fixed allele differences were found and none of the polymorphic loci showed a significant difference in allele frequency between the two morphotypes. Generally, smaller (<45 cm standard length) per adult fish dominated the small-eye morphotype. Sexually mature or ripe males and females were only found among

the larger, big-eye morphotypes. This indicates an age-related shape change occurring at around 40–50 cm standard length. A full discussion of size, shape and genetic variation in blue-eye will be published elsewhere.

Samples of frozen heads and guts received from the New South Wales area included three heads that differed morphologically from the remaining heads. They possessed a blunt snout, a shorter jaw, a relatively larger eye and a narrower head than typical heads of blue-eye trevalla. Routine allozyme analysis identified fixed differences at several loci, indicating that the three heads were from a different species; these three fish were omitted from further analyses. Whole specimens of fish with this differing morphology were subsequently obtained from New South Wales and have been provisionally identified as the related species *Schedophilus labyrinthicus* (Bolch *et al.* 1993).

Allele frequencies and sample sizes for the six areas sampled were recorded (Table 4). Heterozygotes at all loci showed banding patterns consistent with the known subunit structure (e.g. Ward *et al.* 1992). Multiple χ^2 tests were carried out to determine whether allele frequencies differed between repeated collections at the same site. Only *PGDH** differed significantly ($P=0{\cdot}034$) between the two collections at the ETAS site. After applying the Bonferroni adjustment this difference was not significant.

In all, 27 χ^2 tests of goodness of fit to Hardy–Weinberg expectations were carried out. *PEP-2** in the Maatsuyker sample ($P=0{\cdot}047$, $n=79$) and *PGDH** in the Cascade Plateau sample ($P=0{\cdot}048$, $n=67$) showed significant deviation from expected proportions. After applying the Bonferroni procedure for multiple tests, none of the tests was significant at the adjusted level of $\alpha'=0{\cdot}00185$ (where $\alpha'=0{\cdot}05/27$).

The genetic diversity of trevalla, as measured by total heterozygosity (H_T) at the seven polymorphic loci, ranged from 0·491 for *ADH** to 0·071 for *AAT-1**. For the nine other variable loci, H_T varied from 0·055 for *MPI** to 0·004 for *PEP-1** (Table 4). Twenty loci were monomorphic. The mean H_T across all 36 loci was equal to 0·053.

A series of heterogeneity χ^2 tests on allelic frequency variation were performed using the Roff and Bentzen (1989) procedure (Table 3). Each of the 16 variable loci was analysed separately for all samples and the Bonferroni procedure for multiple tests was applied using $\alpha=0{\cdot}05$ and $\alpha'=0{\cdot}0031$. This analysis did not reveal significant heterogeneity for any locus. Similarly, comparison of the two most geographically separated areas, South Australia and New South Wales, failed to show significant heterogeneity ($P>0{\cdot}175$ for all loci, $P=0{\cdot}601$ across all loci).

Minimal stock differentiation between samples was confirmed by the gene diversity analysis (Nei 1973). G_{ST} values were never greater than 0·022 and were less than 0·004 for each of the seven most polymorphic loci (Table 3). Considering the bootstrap analysis, G_{ST} was greater than $G_{ST\cdot null}$ for 8 of the 16 variable loci, but after application of the Bonferroni adjustment for multiple tests, which set $\alpha'=0{\cdot}0031$, no locus showed statistically significant differentiation (Table 3). The mean G_{ST} across all 36 loci was equal to 0·0038, approximately the same as the mean 36-locus value of $G_{ST\cdot null}$ of 0·0044 (s.d. = 0·0011). Thus this estimate of G_{ST} may have arisen solely from sampling error.

Discussion

No genetic distinction could be drawn between the big-eye and small-eye forms of blue-eye trevalla, suggesting that these are two morphotypes of a single species. The change in morphology occurs at about 40–50 cm standard length, and sexual maturity is associated with the big-eye morph. A more detailed analysis of morphology and genotype is in preparation. *Schedophilus labyrinthicus*, provisionally identified among the samples from New South Wales (Bolch *et al.* 1993), is sometimes caught in large quantities by New South Wales fishers although it is currently not managed as a fishery separate from the blue-eye fishery (K. Rowlings, personal communication). No specimens of this species were present

Table 4. Allele frequencies in samples of *H. antarctica* from six areas and (*n*) numbers of fish examined

Abbreviations for areas sampled as in Table 1

Locus	Allele	SA	WTAS	MAAT	ETAS	CASC[A]	NSW
*sAAT-1**	115	0·222	0·220	0·196	0·185	0·179	0·188
	100	0·778	0·780	0·804	0·815	0·821	0·813
	(*n*)	(142)	(143)	(79)	(154)	(67)	(96)
*sAAT-2**	140	–	0·010	–	0·005	–	0·021
	100	1·000	0·990	1·000	0·979	–	0·979
	60	–	–	–	0·016	–	–
	(*n*)	(24)	(50)	(24)	(96)	(0)	(24)
*mAAT**	90	–	0·003	–	–	–	–
	−30	0·046	0·028	0·044	0·039	0·030	0·031
	−100	0·954	0·969	0·956	0·961	0·970	0·969
	(*n*)	(142)	(143)	(79)	(154)	(67)	(96)
*ACP**	100	1·000	1·000	1·000	1·000	–	1·000
	(*n*)	(47)	(50)	(24)	(24)	(0)	(24)
*ADA**	125	0·046	0·084	0·044	0·045	0·045	0·042
	105	0·011	0·007	0·006	0·003	–	0·005
	100	0·852	0·839	0·880	0·870	0·903	0·885
	95	0·063	0·045	0·051	0·062	0·030	0·057
	80	0·028	0·017	0·019	0·019	0·022	0·010
	55	–	0·007	–	–	–	–
	(*n*)	(142)	(143)	(79)	(154)	(67)	(96)
*ADH**	55	–	–	–	–	–	0·005
	−55	0·380	0·427	0·462	0·445	–	0·438
	−100	0·620	0·573	0·538	0·555	–	0·557
	(*n*)	(137)	(143)	(79)	(154)	(0)	(96)
*AK**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(47)	(50)	(24)	(24)	(24)	(24)
*CK-1**	100	1·000	1·000	1·000	0·986	–	1·000
	95	–	–	–	0·014	–	–
	(*n*)	(24)	(50)	(24)	(71)	(0)	(24)
*CK-2**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*CK-3**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*ESTD**	100	1·000	1·000	0·976	1·000	1·000	0·979
	85	–	–	0·024	–	–	–
	75	–	–	–	–	–	0·021
	(*n*)	(24)	(50)	(42)	(71)	(24)	(24)
*FH**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*G3PDH-1**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)

Table 4 (*continued*)

Locus	Allele	SA	WTAS	MAAT	ETAS	CASC[A]	NSW
*G3PDH-2**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(105)	(24)	(24)
*G6PDH**	100	1·000	1·000	1·000	1·000	–	1·000
	(*n*)	(24)	(50)	(24)	(47)	(0)	(24)
*GAPDH-1**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*GAPDH-2**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*GPI-1**	130	–	–	–	0·025	–	–
	100	1·000	1·000	1·000	0·958	1·000	1·000
	65	–	–	–	0·004	–	–
	(*n*)	(24)	(50)	(24)	(120)	(24)	(24)
*GPI-2**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(120)	(24)	(24)
*IDDH**	100	1·000	1·000	1·000	1·000	–	1·000
	(*n*)	(24)	(50)	(24)	(47)	(0)	(24)
*sIDHP**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*mIDHP**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*LDH-1**	100	1·000	1·000	1·000	1·000	–	1·000
	(*n*)	(24)	(50)	(24)	(47)	(0)	(24)
*LDH-2**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(71)	(24)	(24)
*LDH-3**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(71)	(24)	(24)
*sMDH-1**	130	–	–	0·021	–	–	–
	100	1·000	1·000	0·979	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(71)	(24)	(24)
*sMDH-2**	100	1·000	1·000	0·979	1·000	1·000	1·000
	0	–	–	0·021	–	–	–
	(*n*)	(24)	(50)	(24)	(71)	(24)	(24)
*ME1**	100	1·000	1·000	1·000	1·000	1·000	1·000
	(*n*)	(24)	(50)	(24)	(47)	(24)	(24)
*MPI**	115	0·045	–	–	0·005	–	–
	100	0·933	0·990	1·000	0·979	–	0·958
	85	0·022	0·010	–	0·016	–	0·042
	(*n*)	(67)	(50)	(24)	(95)	(0)	(24)

Table 4 (*continued*)

Locus	Allele	SA	WTAS	MAAT	ETAS	CASC[A]	NSW
*ODH**	120	0·015	0·010	–	–	–	0·021
	100	0·955	0·990	1·000	1·000	–	0·958
	70	0·030	–	–	–	–	0·021
	(*n*)	(67)	(50)	(24)	(47)	(0)	(24)
*PEP1**	100	1·000	1·000	1·000	0·989	1·000	1·000
	85	–	–	–	0·011	–	–
	(*n*)	(24)	(50)	(24)	(95)	(24)	(24)
*PEP2**	105	0·021	0·028	0·032	0·023	0·037	0·021
	100	0·838	0·888	0·848	0·847	0·881	0·859
	90	0·137	0·084	0·120	0·127	0·082	0·120
	85	0·004	–	–	0·003	–	–
	(*n*)	(142)	(143)	(79)	(154)	(67)	(96)
*PGDH**	115	0·004	–	–	0·010	–	0·005
	100	0·856	0·829	0·810	0·812	0·866	0·807
	65	0·141	0·168	0·190	0·179	0·134	0·188
	(*n*)	(142)	(143)	(79)	(154)	(67)	(96)
*PGM-2**	500	0·004	–	–	–	–	–
	350	0·039	0·014	0·038	0·029	0·052	0·016
	100	0·947	0·983	0·962	0·958	0·918	0·984
	~150	0·011	0·003	–	0·013	0·030	–
	(*n*)	(142)	(143)	(79)	(154)	(67)	(96)
*SOD1**	100	1·000	1·000	1·000	1·000	–	1·000
	(*n*)	(24)	(50)	(24)	(24)	(0)	(24)
*XDH**	100	1·000	1·000	1·000	1·000	–	1·000
	(*n*)	(24)	(50)	(24)	(47)	(0)	(24)

[A] No livers or eyes available for Cascade Plateau samples.

in the 142 fish examined from South Australia or in the 443 fish from Tasmania (including Cascade Plateau), although anecdotal evidence indicates that it is very occasionally caught off Tasmania. The extent and nature of this resource requires further investigation.

The average heterozygosity of blue-eye trevalla at about 5% per locus is typical of fish in general (Ward *et al.* 1992) and marine fish in particular (Smith and Fujio 1982; Ward *et al.* 1993). Marine fish frequently show low levels of genetic subpopulation differentiation, but the value of G_{ST} recorded here for trevalla (a maximum of 0·0038) is an order of magnitude less than the average of 57 species of marine teleosts previously studied (0·062, Ward *et al.* 1993). The survey in the present study detected no evidence of genetically isolated subpopulations in the southern Australian region, although only a small portion of the geographic range of blue-eye trevalla was covered. Samples from seamounts off the New South Wales coast could not be distinguished from Tasmanian samples or from a sample from the Great Australian Bight. A G_{ST} value of 0·0038 would equate to an N_em value of around 65 migrants per generation per locality but, given that this G_{ST} value can be accounted for simply by sampling error, numbers of migrants are likely to be much higher than this. It appears either the gene flow between areas is sufficient to maintain a very high degree of subpopulation homogeneity, or that divergence among trevalla subpopulations is recent and too limited to be detected by the present study.

A similar lack of genetic stock structure following allozyme electrophoresis of trawled fish species in the south-eastern region of Australia has been reported for the blue grenadier (Milton and Shaklee 1987), orange roughy (Elliott and Ward 1992) and jackass morwong (Richardson 1982; Elliott and Ward 1993). However, mitochondrial DNA analysis has discriminated between New South Wales roughy and those in more southerly Australian waters (Smolenski *et al.* 1993), and both allozyme and mitochondrial DNA data showed significant differences between Australian and New Zealand samples of morwong (Elliott and Ward 1994; Grewe, Smolenski and Ward, unpublished). It remains possible that subpopulations of blue-eye trevalla may be differentiated by mitochondrial DNA studies.

The locations of blue-eye trevalla spawning areas in Australian waters are unknown although, around Tasmania, mature fish are seen on the east coast between January and March (P. Baelde, Division of Sea Fisheries, Tasmania, personal communication). The reproductive biology and development of the blue-eye trevalla is so poorly understood that the reasons underlying the genetic homogeneity of the trevalla in south-eastern Australia remain unclear; the homogeneity could result from larval, juvenile or adult migration, or from a combination of all these processes.

Prior to this study, the only information concerning stock structure in blue-eye trevalla was gained from short-term tagging studies carried out in New Zealand waters (Horn 1989). This showed that 36 out of 40 tagged and recaptured specimens were caught on the grounds on which they were tagged, although one fish moved 490 km in 137 days and a second fish moved 450 km in 231 days. Horn (1989) concluded that although most individuals are fairly sedentary in the short term (6–8 months), the species is capable of long and fairly rapid migrations; these may be a significant vector for gene flow between subpopulations in Australian waters.

Despite indications from this study that there is significant gene flow between Australian subpopulations, the apparently sedentary nature of most individuals and slow growth rate of adult fish (>2 years of age) raises the potential for depletion of local fishing areas. Australian stocks currently appear to be heavily exploited, as evidenced by the declining average size of fish (Webb 1979; Williams 1993); localized depletion has almost certainly occurred in some fishing areas (Williams 1993). Clearly, management of the blue-eye trevalla fishery should closely monitor current and future fishing effort.

A reliable assessment of biomass is urgently required to assess sustainable yield. Data gained from tagging studies and general biological information on age, growth and mortality would allow more reliable interpretations to be drawn from the present genetic study. In the future, trevalla samples will be examined from Western Australia, New Zealand and further afield to assess the extent of gene flow between more geographically isolated populations.

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Appendix 3

More trevalla meet the eye

The blue-eye or deep-sea trevalla (Hyperoglyphe antarctica) is one of the premium table fishes caught off southern Australia. The fishery currently lands about 800 tonnes a year with a market value of more than $3.6m. Fisheries scientists report that, during a current industry-funded project to investigate the stock structure of this valuable species, a second trevalla species was identified.

Blue-eyes belong to the trevalla family (Centrolophidae). About a dozen trevalla species, including the commercially important warehous (*Seriolella* species), are thought to live in Australian waters, but very little is known about their biology and distribution south of the equator.

The blue-eye was, until recently, thought to be a single widespread species found near land or over seamounts in temperate parts of the Southern Hemisphere.[1] To find out its stock structure, the Fishing Industry Research and Development Council (FIRDC) funded CSIRO to make a genetic study of the blue-eye in south eastern Australian waters.

More than 700 tissue samples were collected with the assistance of hook fishermen, processors, and State Fisheries agencies in Tasmania, South Australia and NSW. Fish were sampled from catches off South Australia around to the seamounts off NSW, including shelf areas and seamounts south of Tasmania. The samples were analysed by protein electrophoresis, which shows inherited differences in individual proteins. The results indicated that only a single genetic stock is present in the region.

At the same time, the scientists investigated fishermen's and scientists' reports that there are two forms of blue-eye, with different eye sizes, body shapes and colours. However, the two forms were genetically indistinguishable. The differences appear to be related to the fish's life history. It is thought that the young fish live a pelagic life until about 45 cm in size, after which they move to a demersal habitat at depths of 200–500 m. During this transition they change slightly in shape and colour (from a dark blue above with a silvery belly to a more uniform blue/brown) and their eye increases in size in relation to body size.

Figure 1. Genetic analyses of the muscle proteins GPI (left) and LDH (right) from ocean blue-eye (lanes 3 and 6) and common blue-eye (all remaining lanes).

A large eye is beneficial in deeper water where light levels are low. Some other adult deep-water fishes also have relatively large eyes (for example, oreos). It is, however, unusual for a fish's eye to grow at a greater rate than its body. In fact the eyes of most higher animals, including fish, decrease relatively in size during growth.

The scientists made an unexpected but important discovery. While extracting tissue samples from 96 blue-eye heads collected on the Taupo and Barcoo seamounts off NSW by Mike Rowley (Fortuna Fishing Company), it was noted that three of the heads had a different shape from the rest. When they were examined genetically, they were found to have several different proteins from the others (see Figure 1).

On examination of further material it was found that this second type resembled the common blue-eye in general appearance but differed in fin-ray counts, and in head and dorsal-fin shapes (Table 1). The simplest way to distinguish these species is by the relative size of the mouth (Figures 2 and 3). Clearly they were another species. The second species, which we have called the 'ocean blue-eye', has been provisionally identified as *Schedophilus labyrinthicus*.

This species has been found outside Australian waters under other scientific names but few adults have been recorded from the Pacific Ocean. NSW fishermen have reported that the ocean blue-eye is sold with its more common relative in the Sydney fish market. Significant catches have been made on the continental slope and seamounts off NSW but few have been caught by Tasmanian fishermen. It may, therefore, prefer warmer waters.

Most trevallas are widely distributed along continental slopes or in the open ocean and only a few species are coastal. Some, such as the common blue-eye, appear to aggregate near the bottom over the continental slope (that is, 200–500 m). Juveniles of *S. labyrinthicus* are planktonic in the open ocean, so the adults may be widely distributed in temperate latitudes of the southern hemisphere.[2] So far, this species is known from the Pacific Ocean between Australia and the Easter Islands, including Lord Howe Island and the Gascoyne Seamount off NSW.[3]

A trevalla identified recently by Japanese biologists[4] from New Zealand as an Atlantic trevalla, *Hyperoglyphe moselii*, also appears to be this species, and an adult specimen was speared by a diver in surf off the North Island of New Zealand.[1] Similar fishes, known by other names, from the islands of the central south Atlantic (Tristan da Cunha) and Indian Oceans (St Paul) may also be this species.

NSW scientists will collect biological information on the ocean blue-eye during a forthcoming study of the NSW dropline fishery, and scientists at the CSIRO Division of Fisheries plan to compare all closely related Australian trevallas to establish its correct scientific name.

About 10 additional specimens of ocean blue-eye are required to assist this work. Any fish smaller than 40 cm long

[1]McDowall, R.M. (1982). The centrolophid fishes of New Zealand (Pisces: Stromateoidei). *Journal of the Royal Society of New Zealand* **12**, pp 103–142.

[2]Ahlstrom, E.H., Butler, J.L. and Sumida, B.Y. (1976). Pelagic stromateoid fishes (Pisces, Perciformes) of the eastern Pacific: kinds, distributions and early life histories and observations on five of these from the north west Atlantic. *Bull. Mar. Sci.* **26(3)**: pp 285–402.

[3]McAllister, R.E. and Randall J.E. (1975). A new species of centrolophid fish from Easter Island and Rapa Iti Island in the South Pacific. *Natl. Mus. Nat. Sci. (Ottawa), Publ. Biol. Oceanogr.* **8** (ix), p 7.

[4]Amaoka, K., Matsuura, K., Inada, T., Takeda, M., Hatanaka, H. and Okada, K. (1990). *Fishes collected by the R/V Shinkai Maru around New Zealand*. Japan Marine Fishery Resource Centre, Tokyo, Japan.

Ocean blue-eye: more specimens needed!

During an industry-funded study of blue-eye trevalla stocks in Australian waters, scientists found that the northern component of the fishery comprises two distinct species: the blue-eye (*Hyperoglyphe antarctica*) and a newly recognised species, the ocean blue-eye (provisionally identified as *Schedophilus labyrinthicus*).

These species differ from each other in fin-ray counts, head width and jaw size (see Table 1). Although the ocean blue-eye may be common off NSW, its adult form is presently known from only a few individuals world-wide.

More frozen, whole specimens are urgently required so that its correct scientific name can be established. Juveniles of either species are also very rare; any specimens smaller than 40 cm in length would be of great interest to CSIRO and NSW Fisheries Research Institute scientists.

Table 1

Features distinguishing the two blue-eye species

	Ocean blue-eye (S. labyrinthicus)	*Common blue-eye* (H. antarctica)
Dorsal fin	7–9 spines 26–29 rays Spines grading in length evenly into rays (last spine only slightly shorter than first ray)	9 spines 18–21 rays Spines not grading into rays (last spine much shorter than first ray)
Anal fin	3 spines 18–19 rays	3 spines 13–16 rays
Upper jaw	short (barely reaching past front of eye)	long (reaching back to mid-eye or beyond)
Head	Compressed, narrow head with very blunt snout and rounded forehead	Broad, bulky head; larger fish have a flattened forehead; snout blunt to slightly rounded

Figure 2. The common blue-eye or deep-sea trevalla Hyperoglyphe antarctica, *caught south of Geraldton, Western Australia, from 250 m depth (Fish Photographic Collection, CSIRO Division of Fisheries).*

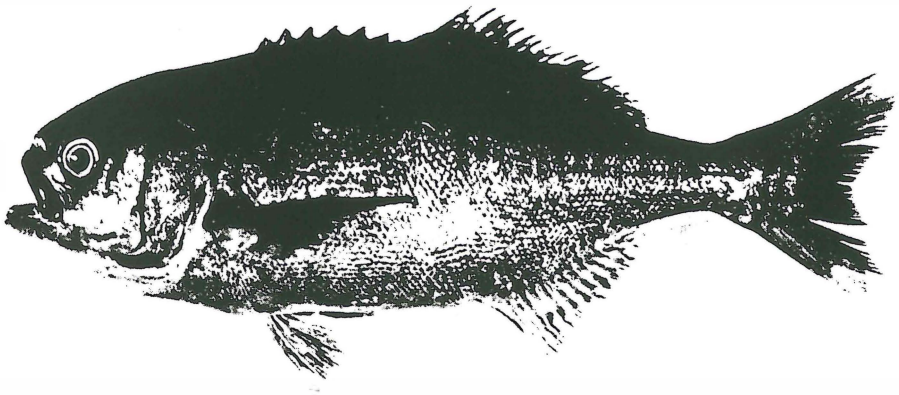

Figure 3. The ocean blue-eye Schedophilus labyrinthicus *caught east of Laurieton, NSW, from 240 m depth (Photograph by Kevin Rowling).*

are particularly valuable, as this species changes shape with age. Also, little is known of its biology, although spawning fish have been caught at the Gascoyne Seamount in July. Any biological observations on size, reproductive condition or food habits, would also be useful to scientists.

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Appendix 4

FISHING INDUSTRY RESEARCH & DEVELOPMENT TRUST FUND
APPLICATION FOR GRANT
1991/92

1. PROJECT TITLE

Population structure of the blue-eye (deepsea trevalla), *Hyperoglyphe antarctica.*

2. KEYWORDS: blue-eye, deepsea trevalla, population structure, taxonomy, genetics, morphology.

3. OBJECTIVES

* To investigate whether the two morphs of blue-eye in southern Australian waters constitute separate species through detailed morphological and genetic comparison of the two identifiable morphs, and to prepare guideline information on their spatial and geographic distributions.

* To assess stock structure and to determine the approximate extent of gene flow among populations in different areas of southern Australia.

4. JUSTIFICATION

(a) The need for research.

The blue-eye (or deepsea trevalla) constitutes a small but important part of the S.E. trawl fishery. Since the discovery of commercially significant quantities off the east coast of Tasmania by Cowper and Downie (1957), and further survey work off western Tasmania (Wilson, 1981), Victoria (Winstanley, 1979) and South Australia (Jones, 1985), the resource has been exploited primarily through the use of droplines in the 100-600 m depth zone. Catches using this traditional method have been relatively stable at a total Australian catch of around 1000 tons (Demersal and Pelagic Fisheries Research Group meeting 28) making it one of the more significant fisheries of the area. Trevalla is the premium marine table fish of Tasmania and is also a high value species in New South Wales and Victoria. Sashimi quality trevalla have recently been exported to Japan from New South Wales.

The fishery is currently embroiled in controversy. With the rapid expansion of the orange roughy fleet off Tasmania, and the closure of roughy fishing grounds for part of the year, some trawler owners have recently diversified their trawling operations to midwater where they have caught appreciable quantities of trevalla. The professional hook-and-line fishermen have urged a ban on midwater trawling for trevalla, fearing that such methods will deplete stocks very rapidly and thus threaten the traditional fishery with extinction. The Tasmanian Minister for Primary Industry, Mr David Llewellyn, has raised the issue with his Federal counterpart, Mr John Kerin, who on November 9, 1990, asked trawl fishermen not to increase their trevalla catches. Since then weight limits for trevalla caught by trawlers have been imposed.

Recent concern over the trevalla fishery means that a management plan for the species is needed urgently. However, very little is known about the resource making a scientifically informed policy difficult to formulate. For example, there are no biomass estimates and it is not known whether the inshore and offshore fishes are different stocks. Indeed, it appears likely that the deepsea trevalla comprises not one, but two species. If the latter is true, catch statistics collected to date may be useless in assessing stock abundance. The two distinct morphs are distinguishable by relative eye size, colour and body shape (Last, unpublished) but their relative abundance, maximum sizes, and their spatial and geographic distributions off southern Australia remain a mystery.

(b) Past and present research

The genus *Hyperoglyphe* Gunther presently contains six species (Haedrich, 1967). Of these, three have been recorded from the Southern Hemisphere: *H. antarctica* (Carmichael), *H. moselii* (Cunningham) and *H. macrophthalma*. The blue-eye, *H. antarctica*, is thought to be widespread in temperate areas with its range including all continents, many islands and some seamounts. The other two species are confined to the Atlantic Ocean although *H. moselii* occurs together with *H. antarctica* off South Africa.

Recently, a second form of blue-eye was discovered in Australian waters. This morph is paler, deeper-bodied, has a less blunt head and proportionally smaller eye than the 'typical' blue-eye. Its eye is also yellowish rather than bluish. It is uncertain if this form is a species distinct from *Hyperoglyphe antarctica* or simply a morph of the latter. The taxonomic relationships of these forms have not been investigated. If these morphs are valid species, it is possible that the scientific name for the blue-eye is being used incorrectly. Interestingly, other scientific names have been applied in the past to blue-eye from Australia, although McDowall (1982), without examining material from Australia, considered these names, *Hyperoglyphe porosa* and *H. johnstonii*, to be junior synonyms of *H. antarctica*.

Stock structure within *Hyperoglyphe* is also unknown. As well as being widespread along the continental slope, populations occur on nearby seamounts. Some, such as the Cascade Plateau, have provided large catches in the past. Stocks on these seamounts may be separate particularly in view of the fact adult trevalla appear to be associated with the continental margin within a rather narrow depth band. Thus, while they are taken well above the bottom by midwater trawlers near the shelf break, they have not been caught by foreign trawlers in the open ocean. The stock issue is an important management consideration.

The location and dispersal patterns of juvenile blue-eye remain a mystery. Juveniles of other members of the family, such as the warehou's (*Seriolella* spp) are found commonly off southern Australia during the spring in association with jellyfish. While juveniles of the closely related *H. perciforma* occur in large numbers off New England under flotsam during the summer (Haedrich, 1967), juveniles or larvae of Australian members of the genus have not been collected. This is despite substantial larval sampling in recent years.

* This proposal has been discussed with Dr Howel Williams of the Division of Sea Fisheries, Tasmania, who is applying to the Fishing Industry Research Council for a grant to assess the trevalla fishery and to analyse the impact of midwater trawling. The two proposals are complementary, and while it would certainly benefit our proposed work if all or part of Dr Howel's proposal was funded, the successful outcome of our project is not dependent on such funding.

* This proposal has been considered, in outline form, by the December 1990 meetings of DPFRG and SETMAC, and was endorsed by both bodies.

(c) Benefits to the fishery

Better knowledge of species and population structures will lead to better informed management policies and an increased probability of a sustainable fishery. Basic biological information is lacking for the trevalla, and it is important that this research either precedes or is carried out at the same time as more detailed studies of the fishery.

(d) Performance criteria

The success of the proposal may be evaluated by its success in settling the one species/two species controversy and by its success in providing information on stock structure. This will lay the basis for any future stock assessments or biological studies of the species.

5. PROPOSAL IN DETAIL

(a) Plan of operation

(i) Method of procedure

Samples will be collected from a variety of sources. These will include the RV "Southern Surveyor" on primarily orange roughy cruises to St Helen's Rise (N.E. Tasmania) in July 1991 and to Maatsuyker (S. Tasmania) in January 1992, from commercial fishermen (both from dropliners and trawlers), and through collaboration with state fisheries organizations. All samples will have to be fresh or frozen.

For part of the proposed work (eg describing the two morphs), whole fish will be required, while for other parts (eg stock discrimination) it is anticipated that only heads and livers will be needed. Heads and livers will be obtainable at no cost from fish processors, while whole fish will either have to be purchased and/or obtained through RV "Southern Surveyor" or state fisheries. Australian samples will be collected from around Tasmania, from Victoria and New South Wales, and from West and South Australia. Samples from more distant regions will be analysed for comparative purposes. Dr Peter Smith, of MAF Fisheries, Wellington, has agreed to supply blue-eye from New Zealand, and Dr Rob Leslie (Environmental Affairs, CapeTown) will supply material from southern Africa.

Description of the two morphs.

The two morphs will be examined using classical taxonomic techniques supplemented by electron microscopy and osteological dissection if necessary. Variation in morphometric traits will be assessed using a video camera linked to a microcomputer running shape analysis software (MORPHOSYS). Otolith structure has been used to distinguish other members of the family (Karrer, pers. com.; Agafonova, 1986) and may be useful in this study. Variation in meristic traits such as fin ray counts, gill rakers and vertebral numbers (using X-ray photography) will also be recorded. Results will be assessed using multivariate statistical procedures.

Genetic analysis to determine species status will involve the description of the products of forty or more protein coding loci revealed through the use of starch and cellulose acetate gel electrophoresis of muscle, liver, heart and eye tissues. Gene frequencies in the two morphs will be estimated and compared statistically: if the two morphs are two species then they should show significant differences in allele frequencies and probably several loci will be species-diagnostic. If such loci are found, any hybrids would be immediately identifiable from the allozyme data as such individuals would be multiply heterozygous for all diagnostic loci. If there are no detectable genetic differences between the two morphs, then the conclusion would be that the morphological variation is consistent with intra-specific variation.

Stock discrimination.

All fish will be analysed electrophoretically for all loci identified as polymorphic in a preliminary screening of one hundred fish taken from a number of different locations. Gene frequencies from different areas will be compared statistically and use will be made of the BIOSYS-1 software package to estimate the genetic distances between operational taxonomic units (species and/or subpopulations). Significant differentiation among samples within species implies a reduction in gene flow among such samples and indicates that for management purposes it would be wise to recognise such samples as originating from separate stocks. Repeat samples will be taken from one or two locations to assess the extent, if any, of inter-seasonal genetic variability.

Evidence from allozyme analysis of subpopulation structuring in Australian fish has been found for some species (jack mackerel, Richardson, 1982; narrow-barred Spanish mackerel, Shaklee, in prep., barramundi, Salini & Shaklee, 1988; snapper, Johnson et al., 1986; yellowfin tuna, Smith et al., 1988; eastern school whiting, Dixon et al., 1987) although not all (three species of sharks, Lavery & Shaklee, 1989; MacDonald, 1988; jackass morwong, Richardson, 1982; blue grenadier, Milton & Shaklee, 1987; orange roughy, Ward & Elliott, unpublished).

(ii) Facilities available

The CSIRO Division of Fisheries (Hobart) has the following facilities available:

. Modern laboratories, well equipped with the many capital items required for a biochemical genetics programme, including freezer rooms, ultra-cold freezers, centrifuges (high speed and micro models), power packs and electrophoresis chambers.
. CSIRO Fish Collection
. Office space
. Use of the services of the RV "Southern Surveyor"
.Administrative support
. Library services
. Excellent micro and minicomputer facilities

(b) Support data

(i) Previous work in this or Related Fields

Dr Ward joined CSIRO Fisheries in January 1990 from Loughborough University, U.K., and is currently carrying out an appropriation and GITLC funded study into morphological and genetic variation in Australian samples of the orange roughy. He is an experienced biochemical geneticist who for the last twenty years or so has used allozyme-based

techniques in analyses of the population structure of a variety of invertebrate and vertebrate species, and has published about 60 papers in this area, including a general review (Ward, 1989). Recent work includes studies of stock structure, hybridisation and phylogeny in several groups of freshwater fishes (Billington et al., 1988, 1990; Bodaly et al., 1989, 1990; Ward et al., 1989, and studies of the phylogenetic relationships of a number of European species of the intertidal molluscan genus *Littorina* (reviewed in Ward, 1990).

Dr Last has been employed since 1977 as a fisheries biologist and taxonomist. Since joining CSIRO in 1985, he has acted as the taxonomic consultant to Division of Fisheries field programs, during which time he has identified fishes from representative marine habitats Australia wide. He has published widely on fish taxonomy (recent articles include Last, 1987; Last & Edgar, 1987; Last & Gomon, 1987; Francis et al., 1988; Gomon & Last, 1987a,b; Kishimoto et al., 1988.; Pavlov et al., 1988) and was coauthor on a guide to the fishes of Tasmania (Last et al., 1983) as well as contributing to a number of other guides to Australian fishes (eg Edgar et al., 1982; Last and Stevens, in press). He is also a coauthor to a major guide to Australian sharks and rays which will soon be published.

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6. RESEARCH PRIORITY

This proposal falls into two of the research priority areas recognised by FIRDC:

* Fish Resource Assessment - biological
* Advancement of Fisheries Science - stock separation

7. TRANSFER OF RESULTS TO INDUSTRY

Results will be presented to the Demersal and Pelagic Fisheries Research Group, the Australian Marine Science Association and the Australian Society for Fish Biology. A report of the findings will be given to SETMAC and papers published in Australian Fisheries and international journals.

8. PREDICTED COMMENCEMENT AND COMPLETION DATE

Commencement date: July 1 1991
Completion date: June 30 1992

Duration of Project: one year

9. REQUESTED BUDGET

item	1991/1992
Salaries & Wages	$37,129
Operating Expenses	$11,000
Travel Expenses	$1,000
Capital Items	none
TOTAL	$49,129

10. FUNDS SOUGHT FROM OTHER SOURCES

none

11. FINANCIAL CONTRIBUTION OF APPLICANT

	$
(a) Salaries of Existing CSIRO Staff (including overheads)	
1 CSOF7 (Ward, 25% of time)	14,000
1 CSOF6 (Last, 15% of time)	7,097
1 CSOF5 (Elliott, 25% of time)	11,185
Subtotal	32,282
Superannuation	5,940
Comcare	807
Leave loading	325
Total Salaries	39,354
(b) Operating Expenses	nil
(c) Capital Cost of Equipment Available	
Genetics Laboratory	130,000
Morphometric equipment	5,000
Freezer room storage	+
Fish Taxonomy Laboratory & Collection	60,000
Car	15,000
Total Capital	210,000+

12. DETAILED BUDGET

Salaries

Name: to be appointed	
Position: CSOF3	
Salary/Wages	30,297
On-costs.....(i) Superannuation	5,575
(ii) Comcare	757
(iii) Leave loading	500
Total Salaries	37,129

Travelling Costs

fares	none
allowances) 9 days travel @ $91/day*	819
accommodation)	
vehicle costs (petrol,oil)	181
other (specify)	none
Total Travel	1,000

* This travel is needed in order to visit ports around Tasmania for sample collections.

Operating Costs

Helena Titan III cellulose acetate gel plates: 10 packs of 100/pack @ $350/pack	3,500
Enzyme substrates, dyes, cofactors, coenzymes,starch	2,000
Miscellaneous expendables (eg microtubes, pipette tips, buffer chemicals, storage bags, X ray film, bone stains)	1,500
Fish purchases	2,000
Freight costs	2,000
Total Operating Costs	11,000

Capital Costs

none

Total Proposed Expenditure	**$49,129**
Date of compilation of financial data	**10/12/90**

13. ORGANIZATION

Head Responsible for Project: Dr P.C.Young (Chief)
Name of Organization: CSIRO Division of Fisheries
Address: GPO Box 1538
Hobart Tasmania 7001

Telephone: (002) 206 222 Fax: (002) 240 530 Telex: 57182

14 PROJECT SUPERVISORS

Names: Dr Robert Ward (Principal Research Scientist)
Dr Peter Last (Senior Research Scientist)

Address: CSIRO Division of Fisheries
GPO Box 1538
Hobart Tasmania 7001

Telephone: (002) 206 222 Fax: (002) 240 530 Telex: 57182

15 STAFF INVOLVED ON PROJECT

			Time spent on project	
			FIRDC	Divisional
Robert D Ward	PhD	Project Co-Supervisor	-	25%
Peter R Last	PhD	Project Co-Supervisor	-	15%
Nicholas G Elliott	PhD	Laboratory Scientist	-	25%
to be appointed	-	Technical Officer *	100%	-

* To assist with the collection of samples and to carry out much of the morphometric and genetic laboratory work).

16 ADMINISTRATIVE CONTACT

Name: Peter Green (Divisional Finance Manager)
Address: CSIRO Division of Fisheries
GPO Box 1538
Hobart Tasmania 7001

Telephone: (002) 206 222 Fax: (002) 240 530 Telex: 57182